Physiological characters underpinning cultivar differences in spear yield of field-grown asparagus (*Asparagus officinalis* L.)

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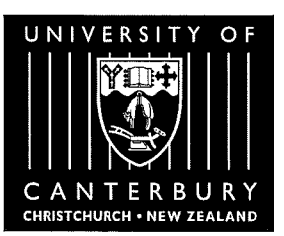

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This thesis is dedicated to my parents,

Qixiu Guo and Xie Zhang

provided me with an unparalleled life experience,

my wife and my daughter

Hong Zhang and Belinda Bei Guo

whose inspiration never cease

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LIST OF ABBREVIATIONS

A	net photosynthetic rate
A_{max}	light saturated net photosynthetic rate
A_{sat}	light and CO_2 saturated net photosynthetic rate
AI	acid invertase
C_a	atmospheric CO_2 concentration
C_I	intercellular CO_2 concentration
DW	dry weight
FW	fresh weight
g_s	stomatal conductance
J_{max}	potential rate of RuBP regeneration
L_{stom}	relative stomatal limitation on photosynthesis
NI	neutral invertase
R_d	dark respiration rate
RGR	relative growth rate
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
SLW	specific leaf weight

SPS	sucrose phosphate synthase
SS	sucrose synthase
TNC	total non-structural carbohydrate
V_{cmax}	maximum rate of carboxylation
V_{lim}	substrate limited SPS activity
V_{max}	substrate saturated SPS activity

ABSTRACT

Although it has long been recognized that genetic variation in spear yield in asparagus (*Asparagus officinalis* L.) is related to the amount of storage carbohydrate reserve in the storage roots, which in turn is linked to photoassimilate production in the previous season, the physiological basis for this variation is not known. In this study, diurnal and seasonal changes in photosynthetic parameters, carbon partitioning parameters and carbon utilization in developing spears were investigated in two asparagus cultivars with contrasting yield. The purpose of the investigations described in this thesis was to characterize the physiological characters underlying cultivar differences in spear yield of asparagus, with the emphasis on carbon assimilation, partitioning, utilization and sucrose metabolism during an annual cycle.

Seasonal patterns in photosynthetic parameters were strongly dependent on cladophyll developmental stage in both cultivars. The greatest photosynthetic rates (A) of 8.94 ± 0.54 μmol m^{-2} s^{-1} for the high-yielding cultivar (ASP-69) and 6.50 ± 0.38 μmol m^{-2} s^{-1} for the low-yielding cultivar (ASP-03) were observed in fully expanded cladophyll tissue measured in mid-summer (February) when both photon flux density (PFD) and temperature were at a maximum. A significant decline in A was measured in April. This was accompanied by a significant decrease in both stomatal conductance (g_s) and ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) activity. A close correlation between A and g_s (r = 0.84) was observed. Although a correlation between A and total rubisco activity did not exist, both *in vivo* and fully activated rubisco activities in ASP-69 were significantly greater than in ASP-03, indicating the important role of this enzyme to cultivar differences in photosynthetic capacity. The difference in photosynthetic capacity between the two cultivars was related to significant differences in cladophyll thickness and specific leaf weight (SLW).

Maximum photosynthetic rate (A_{max}) was positively correlated with sucrose phosphate synthase (SPS) activity (r = 0.86). ASP-69 exhibited greater SPS activity and sucrose content than ASP-03 in fully expanded and mature cladophyll tissue. ASP-69 also displayed a higher percentage of soluble sugar in stem cell sap than did ASP-03. These results suggest that carbon translocation rate in ASP-69 is higher than in ASP-03. Sucrose synthase (SS) activity in storage roots in ASP-69 was significantly greater than in ASP-03 during fern growth season. Total non-structural carbohydrate (TNC) concentration in storage roots did not differ in the two cultivars. Biomass analysis revealed that ASP-69 had a greater root/shoot ratio than in ASP-03, suggesting that the total carbohydrate storage pool rather than carbohydrate concentration is an important determinant of asparagus yield.

Rates of assimilate export estimated from *A* and dry mass changes were highest at midday and coincided with maximum assimilation rate in both cultivars. A positive correlation was found between *A* and assimilate export rate (r = 0.87) and this relationship did not differ between the two cultivars studied. The greater carbon export rate per unit cladophyll area measured in ASP-69 was associated with significantly higher *A* and sucrose concentration in the cladophyll tissue in comparison to ASP-03. Biochemical evidence indicated that the greater *A* and sucrose accumulation in ASP-69 were associated with a significantly higher SPS activity ($P < 0.05$). Phloem ^{14}C exudate analysis confirmed the results estimated by dry mass changes and revealed that ^{14}C flux out of cladophyll tissue in ASP-69 was significantly greater than in ASP-03.

The greater spear elongation rate measured in ASP-69 was associated with a significantly higher hexose accumulation ($P < 0.05$) in spear tissue in comparison to ASP-03. However, sucrose content was similar in the two cultivars, suggesting more efficient machinery for transport and catalysis of carbohydrate in spears of ASP-69. Biochemical evidence indicated that the greater elongation rate in ASP-69 was associated with a significantly higher acid invertase (AI) activity ($P < 0.05$) in the

elongation zone, whereas SS activity was not significantly different between the two cultivars. There was little neutral invertase (NI) activity detected in either cultivar. These results strongly suggest that it is AI and not SS or NI that is an important determinant of the difference in sucrose metabolism between the two asparagus cultivars in metabolising imported sucrose in the elongation region, which in turn plays a part in regulating the import of sucrose into spear tissue. The profile of sucrose cleaving enzyme activities along spear sections indicated that SS was the dominant enzyme in both tip and base of spears, whereas AI was the dominant enzyme in the elongation zone. Overall the data substantiate the conclusion that changes in activities of sucrose cleaving enzymes are correlated with sink functions in developing spears.

The results obtained from this study are consistent with a feed-forward relationship among photosynthesis, sucrose synthesis and assimilate export in the cladophyll tissue. Both metabolic and anatomical factors appear to play significant roles in determining differences in photosynthetic capacity between the two asparagus cultivars studied. For the role of carbohydrate storage roots, it is the pool of total carbohydrate storage rather than carbohydrate concentration that is an important determinant of asparagus yield. This was indicated by the fact that high-yielding cultivar (ASP-69) exhibited a high percentage of young roots to the total biomass than the low-yielding cultivar (ASP-03). This difference was related to a great SS activity in ASP-69. In developing spears, ASP-69 displayed great sucrose cleaving enzyme activities than in ASP-03, indicating that carbohydrate demand in the sink tissue is an important determinant of spear development.

The overall results substantiate the conclusion that spear yield is influenced by both source and sink properties, in which spear elongation is closely related to spear ability to import carbon and the overall yield is determined by the available carbohydrate reserve accumulated in the carbohydrate storage pool, which in turn is linked to assimilate production.

CHAPTER 1

INTRODUCTION, REVIEW OF LITERATURE AND RATIONALE

Chapter 1

Introduction, review of literature and rationale

1.1 Introduction

Asparagus (*Asparagus officinalis* L.) has an established place on world food markets. It has been estimated that some 218,335 hectares were grown worldwide in 1997 (Benson 1999). Maximising the productivity of cultivars has been the subject of extensive research [Nichols, 1990; Benson, 1999). However, increased efforts in breeding practice have concentrated on using agronomic characters, such as yield or survival, as the selection criteria, without a sound understanding of the underlying physiological mechanisms (Mullen *et al.* 1993; Paschold *et al.* 1993; Scholten and Boonen 1993; Faville *et al.* 1999b).

Agronomic criteria currently offer the most integrated approach to develop selection and breeding programs in most species. These are most successful if selection and breeding approaches are linked to the relevant physiological mechanisms or processes limiting to crop yield (Gifford and Evans 1981; Boble and Rogers 1992; Evans 1994; David 1995; Lawlor 1995; Daie 1996). Recent studies have suggested that genetic variation in photosynthetic capacity among asparagus cultivars may partly contribute to cultivar differences in spear yield (Woolley *et al.* 1996; Bai and Kelly 1999; Faville *et al.* 1999b). This phenomenon may be related to an index of genetic preference in

assimilate partitioning into storage roots, as the amount of storage root carbohydrates and spear yield is commonly closely coupled (Ellison *et al.* 1960; Benson and Takatori 1980; Haynes, 1987; Faville *et al.* 1999a; Wilson *et al.* 1999). To get a full understanding of the physiological basis underpinning cultivar differences in spear yield, photosynthesis, assimilate translocation and partitioning need to be treated as integrated processes, since these are linked by numerous interactions (Gifford and Evans 1981; Daie 1985; Daie 1996; Farrar 1996).

The purpose of the investigations described in this thesis was to characterize the physiological parameters underlying cultivar differences in spear yield of asparagus, with the emphasis on carbon assimilation and partitioning and sucrose metabolism during an annual cycle. Details of aims are described further in Section 1.3.

1.1.1 Taxonomy

Asparagus, the common name known in the world food market, is the edible shoot commercially harvested from *Asparagus officinalis* L., a monocotyledonous plant belonging to the family Liliaceae (Tutin *et al.* 1980). Asparagus is normally a dioecious plant producing small insect-pollinated flowers throughout the summer. The small red berry contains up to 6 seeds developing on female plants (Robbins and Jones 1925; Tutin, 1980). Cultivated asparagus is diploid (n = 10) (Ammal and Kaul 1966). *A. officinalis* is a native of Europe and is now grown commercially in temperate and tropical climates throughout the world (Robb 1984; Benson, 1999).

1.1.2 Botanical description

A mature asparagus plant has the habit of an herbaceous perennial, consisting of aboveground shoots and an underground "crown" supported by a mass of fleshy roots (Kidner 1959; Robb 1984). The aerial shoots (ferns) are multi-branched, and can reach a height of up to 2 meters. Each branch is composed of short, leaf-like branches known as cladophylls. The true leaves on the cladophylls have been reduced in size to form very small scales (Mullendore 1935; Robb, 1984). This modification enables moisture loss to be reduced so the plant can survive dry and windy conditions (Toleman 1980). Cladophyll tissue performs the same functions as the leaves of most higher plants. The crown consists of short rhizomes with fleshy, unbranched storage roots and many individual buds from which the edible shoots or spears arise. The storage roots vary in thickness from 2 to 6 mm according to age, nutrient supply and genetic composition. The life of a storage root is approximately three years and is replaced by new ones originating from the base of buds (Blasberg 1932; Robb, 1984). Fibrous roots are attached to the storage roots and are the chief absorbing organs. Buds are present at the growing tip of the underground stem (Blasberg 1932). The crown gradually increases in size with age and the roots act as a carbohydrate storage reserve (Pressman *et al.* 1993). Under ideal conditions, an asparagus plant can live for 10 to 15 years with 10 years of commercial harvest (Kidner 1959; Nichols, 1985).

1.2 Review of literature

1.2.1 Introduction

Crop yield among species or cultivars under field conditions is a complicated process regulated by both genetic and environmental components (Daie 1988; David 1995). Among the environmental components are soil water supply, temperature, nutrient

availability and various cultural practices. Of all the genetic yield determinants, carbon production and its partitioning are considered the major limits to improving crop productivity (Gifford and Evans, 1981; Daie, 1985; Evans, 1993). Differences in the agronomic performance of asparagus plants have been attributed to differences in the amount of carbohydrate reserve in the storage roots produced in the previous season (Fisher 1982; Bai and Kelly 1999; Faville *et al.* 1999a) or the efficiency of partitioning carbohydrates into storage roots (Benson and Takatori 1980; Dufault and Greig 1983; Wilcox-Lee and Drost 1990).

This review deals primarily with source (cladophyll) to sink (storage root) relationships in asparagus. Emphasis is given to those aspects that affect carbohydrate assimilation, partitioning and utilization in relation to spear yield. Sink and source properties in asparagus and environmental effects on both are reviewed first, followed by current knowledge concerning how source and sink activities are coordinated.

1.2.2 Sources of carbohydrates

1.2.2.1 Shoot growth

Shoot growth in asparagus is initiated in the spring from the underground bud. Bud break is controlled by apical dominance within each bud cluster on the underground stem (Tiedjens 1926; Kretschmer and Hartmann 1979). Several plant growth substances influence bud break. The concentration of abscisic acid in the crown buds and root tips is found to be proportional to the degree of dormancy in winter (Matsubara 1980). There is evidence that apical dominance can be suspended by Gibberellic acid when it is applied to spear tips or the underground stem (Kretschmer and Hartmann 1979). Soil temperature, carbohydrate content of the roots and plant age can also exert an impact on bud initiation. An atmospheric temperature of 4.4°C has been used to predict the initiation of bud break in spring (Bouwkamp 1975).

However, field observations indicate that the temperature threshold for bud initiation varies with plant age and cultivars. Young plants often have a lower temperature threshold than old plants (Huang 1979b).

Bud size is closely related to shoot size. Large buds usually produce large shoots when plants are growing in favourable conditions (Tiedjens 1926; Blasberg 1932; Nichols and Woolley 1985; Drost and Wilcox Lee 1997b). However, a decrease in storage carbohydrate reserve may break this balance. Tiedjens (1926) observed that large buds may produce small spears if stored carbohydrate reserve has been depleted. In addition, soil moisture stress in the previous fern growth season decreases the sizes of buds produced (Drost and Wilcox Lee 1997b).

During the spear-harvesting season, shoot growth strongly depends on carbohydrate remobilized from storage roots and atmospheric temperature (Benson and Takatori 1980; Haynes 1987; Wilson *et al.* 1999). Some studies have shown that carbohydrate availability is not limiting during this period, since subsequent fern establishment following spear harvest is a much more severe drain on root carbohydrate (Haynes 1987; Pressman *et al.* 1993; Faville, 1997). Shoot growth rate increases linearly between 7 and 31°C (Culpepper and Moon 1939b; Hughes *et al.* 1990). Unlike most crops, shoot elongation rate in asparagus is greater during the day than during the night (Bluemenfield *et al.* 1961; Robb, 1984). Under favourable growth conditions, shoots are capable of growth rate as high as 30 cm per day (Meyer *et al.* 1960). As the shoot increases in height, the rate of elongation increases rapidly to a maximum at heights between 60 and 70 cm, and then decreases to low values in very tall shoots. The zone with maximum rate of growth is located a short distance below the tip and it is more sensitive to changes in temperature than the zones above and below (Culpepper and Moon 1939b).

As shoot extension growth progresses, the aerial tip, a compact head of short branches covered by scales, extends. Through continued elongation of internodes in the head region and by extensive growth of side branches, a fern-like form is assumed. The small needle-like branches (cladophylls), which are the chief photosynthetic organs of the plant, occur in whorls at the nodes of the stem and branches (Blasberg 1932; Downton and Törökfalvy 1975).

1.2.2.2 Photosynthesis

Asparagus has been described as possessing a C_3 photosynthetic pathway with a relatively high level of oxygen-insensitive respiration (Downton and Törökfalvy 1975). The cladophylls of asparagus are the main site for carbon assimilation although photosynthesis also occurs in all green tissues. Stomata are scattered over the entire surface of the cladophyll (Sawada *et al.* 1962; Downton and Törökfalvy 1975). Subsequent to cladophyll emergence there is a period of rapid transition of the cladophyll from sink to source. This is indicated by the fact that net photosynthesis becomes measurable once the spear starts to assume a fern-like form (Downton and Törökfalvy 1975).

The earliest work on asparagus photosynthesis is reported by Sawada *et al.* (1962) who estimated the assimilation rate by measuring the difference between glucose content from samples collected from the field at different times of the day. They reported that assimilation rate varied during the day from 18 to 33 mg g^{-1} of fresh cladophylls. They also showed that assimilation rate was affected greatly by duration of light period and light intensity. Lin and Hung (1978) measured photosynthetic rate on individual branches at various growth stages using an assimilation chamber. They measured that the net photosynthetic rate was 48.9 mg g^{-1} h^{-1} of fresh cladophylls in one-month old rapidly expanding cladophyll tissue. The photosynthetic rate reached a maximum of 65.3 mg g^{-1} h^{-1} in fully expanded cladophyll tissue (two-month old). In

senescent cladophyll tissue, photosynthetic rate declined to 8.6 mg g^{-1} h^{-1}. In another similar study carried by Downton and Törökfalvy (1975), photosynthetic rate in expanding cladophyll tissue was 17 μmol h^{-1} mg^{-1} chlorophyll, whereas in the fully expanded cladophyll tissue, the net photosynthetic rate was 20.9 μmol h^{-1} mg^{-1} chlorophyll. Recently, Faville *et al.* (1999b) investigated photosynthetic rate of three asparagus cultivars differing in yield on a cladophyll basis. They reported that light saturated rate of photosynthesis (A_{max}), ranged from 3.8 to 6.2 μmol m^{-2} s^{-1} for six-year old field grown plants and 2.4 to 4.8 μmol m^{-2} s^{-1} for nine-month old pot plants. The results indicated that A_{max} was positively associated with asparagus yield among the three cultivars. There was also a highly significant correlation between A_{max} and stomatal conductance (g_s). The variation in photosynthetic capacity was also related to cultivar differences in cladophyll diameter. Estimation of canopy photosynthesis of asparagus has recently been measured by Bai and Kelly (1999) who determined net photosynthesis from whole plants of eight asparagus cultivars in an open infrared gas analysis system. The results showed that net photosynthesis of the eight cultivars ranged from 15.7 to 27.8 μmol m^{-2} s^{-1}. Significant difference ($P < 0.1$) in net photosynthesis were found among the eight cultivars and both yield and specific leaf weight (SLW) were significantly correlated to net photosynthesis.

1.2.2.3 Partitioning of carbohydrate within cladophyll tissue

Carbohydrate accumulation in cladophyll tissue has been investigated in several studies (Haynes 1987; Martin and Hartmann 1990; Pressman *et al.* 1993; King *et al.* 1995). Cladophyll age influences both cladophyll dry weight and the concentration of soluble sugar. As the fern develops in spring, cladophyll dry weight increases. This increase is particularly marked during the period of fern maturation. Cladophyll dry weight then decreases as the fern senesces (Haynes 1987). Seasonal changes in cladophyll dry weight are accompanied by changes in sucrose concentration. King *et al.* (1995) found that concentration of sucrose in cladophyll tissue of field-grown asparagus increased as cladophyll expansion progressed, after which sucrose

concentration remained constant. Sucrose concentration then declined rapidly following onset of cladophyll senescence. By contrast, both glucose and fructose concentration initially declined during cladophyll expansion and then increased with the onset of senescence (King *et al.* 1995). In another study, Pressman *et al.* (1993) reported similar changes in sucrose concentration but different patterns in glucose and fructose concentration. Glucose concentration initially decreased rapidly during the period of cladophyll expansion after which glucose concentration declined steadily in both mature and senescent cladophyll tissue. A similar trend was also observed on fructose concentrations (Pressman *et al.* 1993).

Sucrose is the most abundant sugar present in the mature cladophyll. However, measured concentrations of sucrose have varied in different studies (approximately 140 mg g^{-1} g DW reported by King et al. (1995) and 70 mg g^{-1} g DW reported by Pressman *et al.* (1993)). Published concentrations of glucose and fructose are much more similar at about 20 mg g^{-1} g DW in mature cladophyll tissue. Hexose concentration is less than that of sucrose (Haynes 1987; Pressman *et al.* 1993). There is little information concerning starch accumulation in cladophyll tissue, although it is considered that starch concentration is very low (Pressman *et al.* 1989; Martin and Hartmann 1990).

1.2.3 Sinks for carbohydrate

1.2.3.1 Root growth

Due to difficulties in access, there is much less information available concerning the seasonal growth of the root system. However, agricultural practice has long shown that the optimal spear productivity depends strongly on the size of the root system and the amount of carbohydrate stored in it (Tiedjens 1924; Tiedjens 1926; Kidner 1959;

Benson and Takatori 1980; Shelton and Lacy 1980; Pressman *et al.* 1993; Wilson *et al.* 1999).

The underground root system, or crown as it commonly called, exhibits a branching habit, which, as lateral buds develop, extends the crown in new directions. There is a progressive development of new rhizomes associated with new buds and storage roots are produced during every spring and summer season. The new storage roots are formed at the base of the young, actively growing buds. They grow rapidly and may reach 1 to 2 m in length depending on the environmental conditions (Blasberg 1932; Dufault and Greig 1983; Faville *et al.* 1997). Asparagus prefers a deep soil with good structure (Robb 1984). Although asparagus is a monocotyledon, considerable increase in diameter of the storage roots is considered to be affected through cell division in the cortex. The number of cortical cells in a radial line between endodermis and epidermis varies from 35 to 50 in the older portions of the root, whereas the younger region contains only about 20 to 35 cells (Blasberg 1932). The storage root varies in thickness from 2 to 6 mm according to age, nutrient supply and cultivar. The longevity of a storage root is not definitely known, although lengths of 2 to 4 years have been reported, depending upon growth conditions and genetic composition (Johns and Robbins 1928; Blasberg 1932; Hughes 1992).

1.2.3.2 The physiological role of the storage root

Unlike most vegetable crops, asparagus is a perennial plant and photosynthetic performance does not directly contribute to spear growth (Robb 1984). During the fern growth season, the storage root is the biggest sink for current photoassimilates (Benson and Takatori 1980; Wilson *et al.* 1999). Asparagus plants allocate a significant fraction of their photosynthetic output to long-term storage roots and after winter dormancy storage carbohydrates are utilised in spear growth during the next spring (Benson and Takatori 1980; Shelton and Lacy 1980: Woolley, 1999).

Allocation of photosynthate to long-term storage in the perennial plants fulfils two main ecological functions: (1) support of vegetative re-growth following dormancy (Ho and Rees 1976; Menke and Trlica 1981; Wyka 1999); (2) Survival during the dormant period (Boyce and Volenec 1992; Wyka 1999). It has long been recognised that shoot development in asparagus relies strongly on the carbohydrate reserve in storage roots (Robb 1984). The role of the storage root as a storage organ to support re-growth of shoots in the spring has been used as a tool in agricultural management to achieve increased yield (Huang 1979a; Haynes 1987; Douglas 1990; Feher 1992; Krug 1999a). For example, the balance between length of spear harvest and fern growth season is the key to ensure both maximum spear yield and a full recharge of root carbohydrate reserve which in turn links to the spear yield in the following season (Wilson *et al.* 1999). Extending spring harvest beyond the normal time may prevent the root carbohydrate reserve from being restored to pre-harvest level (Scott *et al.* 1939; Shelton and Lacy 1980; Taga *et al.* 1980; Wilson *et al.* 1999). Several studies have suggested that stored root carbohydrates have a role in survival during low-temperature periods (Pressman *et al.* 1989; Martin and Hartmann 1990; Shiomi 1992; Pressman *et al.* 1993). For example, Pressman *et al.* (1989) have observed an increase in short-chain fructan content in dormant asparagus roots when plants were maintained at low temperature. Similar results have also been reported by Martin and Hartmann (1990) and Shiomi (1992).

The constitution of carbohydrates in the root system shows strong variation during the year. Under temperate climate, fructan content in the root system is relatively stable during the winter dormancy (Scott *et al.* 1939; Shelton and Lacy 1980; Haynes 1987), whereas under Mediterranean climate fructan content declines gradually (Pressman *et al.* 1993). The sucrose content, however, shows an increased pattern until bud break under both climates (Haynes 1987; Shiomi 1992; Pressman *et al.* 1993). Increase in sucrose content is considered as an osmotic buffer involved in frost tolerance (Martin and Hartmann 1990) or as a signal for the sprouting of the dormant buds (Pressman *et al.* 1993).

1.2.3.3 Partitioning of carbohydrate within the storage roots

Seasonal carbohydrate partitioning into the underground crown has been investigated in several studies. Results from those studies indicate that at the fully expanded and mature cladophyll developmental stages, the storage root system is a much larger sink for current assimilate than the rhizome or vegetative bud organs (Haynes 1987; Pressman *et al.* 1993; Faville *et al.* 1999a; Wilson *et al.* 1999; Woolley *et al.* 1999). Although buds have been shown to have a higher priority to store current assimilates, the percentage of assimilate accumulated in buds is much less than the storage roots due to their small mass (Faville *et al.* 1997).

Seasonal changes in carbohydrate concentration in storage roots have been well documented (Scott *et al.* 1939; Shelton and Lacy 1980; Haynes 1987; Pressman *et al.* 1993; Wilson *et al.* 1999). Results obtained from these studies have led to the general conclusion that spears produced in the early spring are largely dependent upon carbohydrate reserve produced during the previous season and stored in the storage roots (Pressman *et al.* 1993; Wilson *et al.* 1999). During the winter dormant period, carbohydrate content in the storage roots does not significantly decrease in a temperate climate (Shelton and Lacy 1980; Haynes 1987). In contrast, a gradual decrease in storage carbohydrate content occurs in the moderate Mediterranean climate, indicating that root metabolism continues to occur in relatively moderate winter conditions (Pressman *et al.* 1993). However, under both climates, an increase in sucrose content during the dormant period is observed (Martin and Hartmann 1990; Shiomi 1992; Pressman *et al.* 1993). The rise in sucrose levels begins in late autumn and continues until sprouting begins in early spring, suggesting that apart from acting as osmotica in response to low winter temperatures, the increased sucrose concentration may have a role as a signal for the sprouting of the dormant bud (Pressman *et al.* 1993). Fructans (fructo-oligosaccharide and fructo-polysaccharide) are the major carbohydrates accumulated in the storage root. Sucrose concentration is lower but shows significant seasonal changes. Both fructose and glucose

concentrations are rather low and relatively constant throughout the season (Shiomi 1992; Pressman *et al.* 1993).

Seasonal patterns of carbohydrate partitioning change with age under field conditions. In the seedling growth stage, the partitioning of assimilates between roots and shoots is initially in favour of shoot production (Fisher 1982; Dufault and Greig 1983). After seedling establishment, partitioning of assimilates is in favour of storage roots (Fisher 1982; Haynes 1987). For a mature plant the root/shoot ratio is relatively stable, balanced by senescence of old storage roots and formation of new storage roots (Tiedjens 1924; Dufault and Greig 1983). Rhizome dry weight increases steadily throughout the growing season and comprises about 8% of the crown dry weight (Fisher 1982; Haynes 1987).

High and low-yielding cultivars appear to differ in partitioning between roots and shoots. A higher root/shoot ratio has been found to be associated with high spear yield (Tiedjens 1924; Wilcox-Lee and Drost 1990; Drost and Wilcox Lee 1997a). Greater root dry weight in high-yielding cultivars may be due to a greater number of storage roots formed (Tiedjens 1924; Benson and Takatori 1980) or early formation in new storage roots and new buds (Dufault and Greig 1983). Certainly, a greater crown biomass will provide a larger carbohydrate pool required during the period of fern establishment. However, it is also evident that carbohydrate concentration can be quite different between the cultivars (Pressman *et al.* 1993).

Using ^{14}C to trace the partitioning of current assimilates from specific ferns of two-year old asparagus at different time of the year, Woolley *et al.* (1999) observed two abrupt changes in the allocation of labelled ^{14}C. The first abrupt change is associated with active new shoot growth, when 70% of ^{14}C is partitioned to the shoot. The second abrupt change occurs in late summer when 74% of ^{14}C is partitioned to the crown (storage roots). A close relationship between dry matter partitioning and ^{14}C

recovered in the storage root was observed. Woolley *et al.* (1999) suggest that the environmental signal inducing a change in partitioning may be decreasing day length. In another similar study using mature asparagus (six-year old) under field conditions, Faville *et al.* (1999) investigated carbon partitioning patterns of three asparagus cultivars with different yield. They found that there was no cultivar difference concerning carbohydrate partitioning patterns between shoots and crown. The majority of ^{13}C was translocated into the underground crown. They concluded that this was not surprising given that there was little new fern growth following labelling. Carbohydrate allocation patterns in the crown appear not to differ among cultivars. The storage root fraction remained the dominant sink for current photoassimilates due to its much larger mass than buds and rhizomes. However, there was evidence that ^{13}C concentrated most strongly in the buds and bud roots (the active, growing end of the rhizomes). During the spear growth season, when buds start to emerge, there is a further remobilization of ^{13}C into new spear growth (Faville *et al.* 1999a). Work conducted by Wilson (pers. Comm.) and Woolley *et al.* (1999) also suggests that different parts of the root system of asparagus may store and supply carbohydrate to the plant at different times of the year. In addition, different carbohydrate pools are utilized at different times and rates during the spear production cycle (Wilson, pers. comm.).

1.2.4 Carbohydrate metabolism

Research into carbohydrate metabolism in asparagus has focused primarily on carbon import into young growing spears, due to their economic importance (King *et al.* 1990; Lill *et al.* 1990; Hurst *et al.* 1993; King *et al.* 1997) and carbon partitioning into the storage roots (Cairns 1992; Shiomi 1992). Carbon metabolism in cladophyll tissue has received much less attention.

Studies on carbohydrate mobilization during spear development have provided good evidence that sucrose is the major carbohydrate translocated from storage roots to developing spears, where it is hydrolysed into hexose and utilised in spear growth (Hurst *et al.* 1993; Irving and Hurst 1993). Based on metabolic status and accumulation of carbohydrates, the critical role of spear tips in controlling spear growth has been identified (King *et al.* 1990; Lill *et al.* 1990). However, the relative roles of sucrose cleaving enzymes in the determination of carbon import and their relationship with spear growth are still not fully understood. Hurst *et al.* (1993) reported that soluble acid invertase activity is four times greater in the middle of the spear than in the tips and bases, whereas sucrose synthase and neutral invertase activities are similar throughout the spear. In contrast, Alam *et al.* (1999) found that sucrose synthase activity is greater in the base than in the tip. In another similar study, Irving and Hurst (1993) observed that sucrose synthase activity is greater than soluble acid invertase in spear tips.

Although the activity of soluble acid invertase is significantly higher in the middle region of the developing spears, there is no correlation found between the activity of soluble acid invertase and hexose concentration along spear sections (Hurst *et al.* 1993). It is considered that the rate of hexose usage, rather than sucrose cleaving enzyme activity, is the main determinant of hexose concentration in developing spears (Hurst *et al.* 1993).

In the storage root tissue of asparagus, fructans are the major storage form of carbohydrates (Shelton and Lacy 1980; Shiomi 1980; Cairns 1992; Shiomi 1992). It is generally known that fructan is synthesized from sucrose via a trisaccharide intermediate, by the concerted action of two distinct fructosyl transferases - sucrose:sucrose fructosyl transferase (SST; EC 2.4.1.99) and fructan:fructan fructosyl transferase (FFT; EC 2.4.1.100) (Cairns 1992). During the fern growth season, increases in fructan concentration in the storage root tissue are closely correlated with

the activities of SST and FFT. Activity of invertase in the storage roots is less variable than SST and FFT, and is highest in the late autumn (Shiomi 1992).

1.2.5 Annual life cycle of a mature asparagus plant

As asparagus is a herbaceous perennial plant, the persistent crown acts as a storage organ, with fleshy roots acting as the plant carbohydrate storage reserve (Kidner 1959). This characteristic gives asparagus an advantage in surviving or coping with harsh conditions, whilst fern growth occurs only during relatively favourable conditions (Robb 1984). In the tropics asparagus ferns can remain active all year (Huang 1979a). In a temperate environment such as New Zealand, asparagus spears emerge in spring from the underground rhizomes when soil temperature reaches about 5°C (Robb 1984). Once above ground, asparagus spears are susceptible to spring frosts, which may limit commercial production during the harvest season. Following the end of harvest in late spring, asparagus spears quickly elongate into asparagus ferns which may grow up to 2 m in height over the summer period (Tiedjens 1924). During the period of fern establishment, shoot growth depends mainly on available carbohydrates from the storage roots until the cladophyll has expanded (Downton and Törökfalvy 1975). During this period, the growth rate of shoots gradually decreases as plant size increases (Tiedjens 1924; Benson and Takatori 1980). This is accompanied by a decrease in remobilization of storage root carbohydrate into the shoots (Benson and Takatori 1980; Nichols and Woolley 1985). The cladophylls of asparagus are the main site of assimilation, although assimilation occurs in all green tissue (Downton and Törökfalvy 1975; Inagaki *et al.* 1989). Following full expansion of the fern and reproduction, the fern senesces and dies in the autumn with the onset of frosts.

The underground crown biomass and content of storage root carbohydrates are relatively constant during winter dormancy. Following spear development and harvest in spring, the storage root carbohydrate content decreases as the reserves are used to

support spear growth (Scott *et al.* 1939; Shelton and Lacy 1980; Wilson *et al.* 1999). During harvest season, carbohydrate reserves are also used in production of new storage roots and new buds (Tiedjens 1924). New buds start in the growing region of a rhizome as scales, which gradually fill out to form individual entities (Tiedjens 1924). Storage root formation is a process associated with bud development into a shoot (Tiedjens 1924; Hughes 1992). After spring harvest, storage root carbohydrate decreases further in support of fern establishment until cladophyll tissue has expanded. The root carbohydrate content is restored to pre-harvest levels by the end of summer, and then generally remains constant or decreases slightly before fern senescence occurs in autumn (Faville 1997; Wilson *et al.* 1999). During the summer fern season, more storage roots and buds are produced. This process is accompanied by senescence of the old parts of the rhizome and attached roots. After winter dormancy, the cycle starts again with the spear emergence from the new buds formed in the previous summer period (Robb 1984).

1.2.6 Prospects for breeding high yield cultivars

Although asparagus has a long history in agriculture, progress in breeding of high yield varieties has not been successful until recent decades (Nichols 1990). In general, male asparagus plants produce larger spears yields than females. This difference between sexes largely explains the superior performance of all-male hybrid cultivars (Ellison *et al.* 1961). Recent breeding efforts have proved that hybrid cultivars have great potential to increase yield (Nichols 1985; Pandita and Bhan 1994; Mullen *et al.* 1996). Unlike the old, multi-parent open pollinated varieties, hybrids are produced from a few parent plants and can be readily improved through parent selection. The hybrids provide more uniform stands of better yielding plant types and have already proved to be capable of producing yield twice those of the old multi-parent varieties. Two hybrid cultivars, Jersey Giant and UC 157, have been widely used to replace the traditional open pollinated varieties. Jersey Giant is an all-male cultivar and has the advantage of not producing seedlings in the field, whilst UC 157 is a single cross

hybrid cultivar containing both male and female plants. Jersey Giant usually produces a relatively higher yield than UC 157 under most environmental conditions (Price and Baughan 1990; Mullen *et al.* 1996). This is probably due to the absence of female function, which removes the problem of unwanted seedling production. More recently, the possibility of using clonal material to improve spear yield has been investigated. It has been shown that some high producing individuals can be used for micro-propagation to produce uniform plant stands with great yield potential (Fraser-Kevern *et al.* 1996; Smeenk *et al.* 1996).

1.2.7 Summary

The body of information on carbohydrate production and partitioning in relation to spear yield in asparagus is impressive. The influences of shoot vigour and dry weight partitioning in the previous season on spear yield in the following season have been studied extensively in breeding efforts. Plant biomass, a measure of the source productivity, is closely linked to spear yield. Increase in yield is positively associated with the amount of storage root carbohydrate reserve which is a direct product of photosynthesis. Photosynthetic performance is positively linked to spear yield. Thus, it is reasonable to assume that source capacity is a major limit to yield in asparagus. Unfortunately, approaches in understanding the mechanisms underpinning such observed cultivar differences in growth and yield have not provided successful explanations to date. A sound knowledge of asparagus physiology, therefore, is needed to provide insight into the physiological factors that determine cultivar difference in spear yield and how it can be manipulated.

1.3 Rationale of the present study

There is evidence that spear yield in asparagus depends strongly on the amount of carbohydrate reserve in the storage roots and the numbers of buds which determine potential spear numbers (Tiedjens 1924; Tiedjens 1926; Scott *et al.* 1939; Ellison and Schermerhorn 1958; Ellison *et al.* 1960; Benson and Takatori 1980; Fisher 1982; Haynes 1987; Pressman *et al.* 1989; Douglas 1990; Pressman *et al.* 1993; Loughton *et al.* 1996; Ernst and Krug 1998; Krug 1999b; Wilson *et al.* 1999; Woolley *et al.* 1999). More recently, some studies have shown that spear yield is positively related to the rate of photosynthesis on both a canopy (Bai and Kelly 1999) and cladophyll area basis (Faville *et al.* 1999b). Since bud number is considered to be genetically controlled (Robb 1984; Drost and Wilcox Lee 1997b), a close relationship among photosynthesis, assimilate partitioning and spear yield in asparagus may be expected, i.e. an increase in photosynthesis should increase the amount of carbohydrate accumulated in storage roots, leading to an increase in yield. In this regard, it may be reasonable to hypothesize that a feed-forward relationship exists between the source (cladophylls) and sink (storage roots) in relation to spear yield (Fig. 1.1). The relationship between source (storage roots) and sink (developing spears) concerning carbon reallocation into spear tissue is largely unclear. However, there is substantial evidence that the sugar and sucrose metabolism status of a sink is instrumental in determining carbon transport from storage source into the growing sink (Ho 1988; Farrar 1992; Hampp *et al.* 1994; Sung *et al.* 1994; Marcelis 1996; Balibrea *et al.* 2000). In this thesis, a hypothesis that sucrose metabolism of developing spears creates a demand to drive carbon import through the phloem is tested.

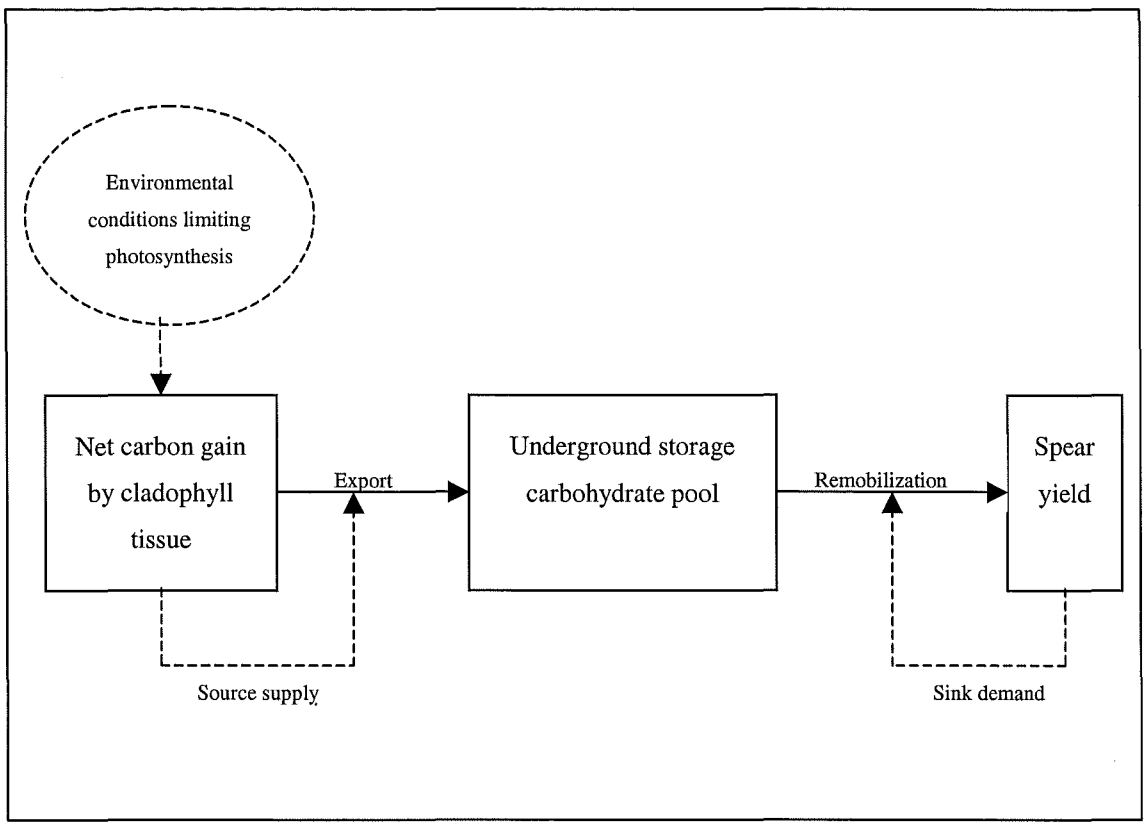


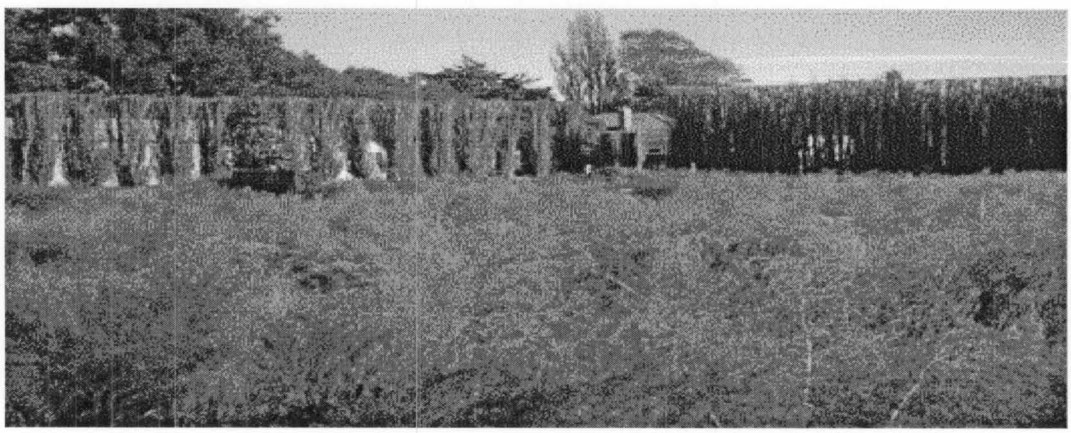
Fig. 1.1. Simplified schematic illustration of the relationship between source (cladophylls) and sink (storage roots) in relation to spear yield.

To date, no attempt has been made to refine or model source-sink interactions in relation to spear yield in asparagus. For the purposes of this thesis, two important questions being addressed were:

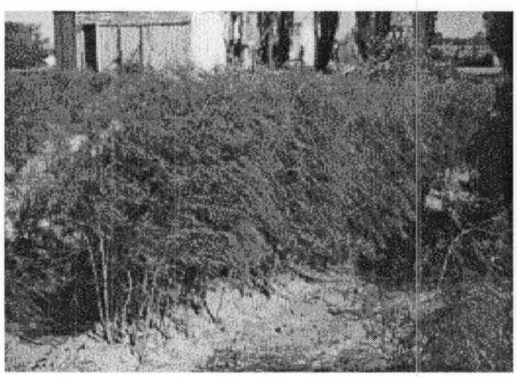
With respect to source-sink relationships in asparagus, what are the physiological limits to carbon mobilization into storage roots and remobilization into developing spears, and within these limits, what metabolic processes contribute to cultivar difference in spear yield?

1.3.1 Study site

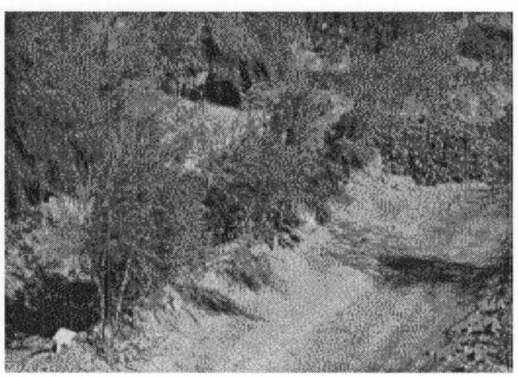
This study was conducted in a pre-existing cultivar trial located at the New Zealand Institute of Crop and Food Research, Lincoln, New Zealand (Plate 1.1). This asparagus trial was established with a split-plot design in September 1992 for production determination purpose.



a. Study site



b. High-yielding cultivar (ASP-69)



c. Low-yielding cultivar (ASP-03)

Plate 1.1. View of study site and cultivars selected: (a) study site located at New Zealand Institute of Crop and Food Research, Lincoln, New Zealand; (b) two all-male clonal cultivars with significant differences in spear size and morphology were selected for this study.

1.3.2 Plant materials

Two asparagus (*Asparagus officinalis* L.) all-male clonal cultivars (ASP-69 and ASP-03) in a pre-existing trial located at the Institute of Crop and Food Research, Lincoln, New Zealand were selected for this study based on differences in spear yield and morphological variations. A significant difference in spear yield between these two selected cultivars had been established for the previous 6 years (W.A. Jermyn, unpublished data). ASP-03 is a low-yielding cultivar with small size of spears and short ferns. By contrast, ASP-69 is a high-yielding cultivar with large spears and ferns in comparison to ASP-03. Three plots (10 plants in each plot) for each cultivar were selected to conduct this study.

1.3.3 Microclimatic conditions

Monthly climatic conditions during the study period, based on meteorological data collected from a local Weather Service Site located 2 km from the study site, are summarized in Table 1.1. The mean maximum and minimum temperatures during January, February and March averaged 22 and 12.6°C, respectively, and in April and May were 16.7 and 6.7°C, respectively. The total monthly precipitation received increased from January to March and then decreased in April and May. The pattern of total solar radiation received decreased consistently from January to May (Table 1.1).

Parameter		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Total solar Radiation	MJ m^{-2}	692	625	478	292	214	161	149	262	417	522	602	763
Total Precipitation	mm	36.2	38.3	56.1	36.3	23.6	69.1	135.1	58	26.6	50.9	60.5	39.1
Mean daily maximum air temperature	°C	22.1	22.2	21.6	16.5	16.8	12.1	10.5	12	14.8	17.3	17.3	18.8
Mean daily minimum air temperature	°C	13.3	12.4	12.2	8.1	5.2	1.8	2.4	2.4	4.4	8.1	9	8.7
Mean daily soil temperature	°C (10 cm)	20.9	20.1	17.2	12.7	9.8	6.6	6.2	6.5	9.6	12.9	14.4	16.5
	°C (30 cm)	20.3	19.8	17.3	13.6	10.5	7.7	6.6	4.8	9.4	12.3	14.2	15.9
	°C (100 cm)	17.7	18	16.9	15	12.4	10.4	8.1	8	9.4	11.2	13.2	14.2
Day/night length	h/h	15.0/ 9.0	13.8/ 10.2	12.4/ 11.6	10.9/ 13.1	9.7/ 14.3	9.0/ 15.0	9.2/ 14.8	10.2/ 13.8	11.6/ 13.4	13.1/ 10.9	14.5/ 9.5	15.5/ 8.5

Table 1.1. Summary of principal climatic factors during the study period based on meteorological data from Lincoln Weather Service Station, Christchurch, New Zealand (2 km from the study site). Both solar radiation (MJ m^{-2}) and precipitation (mm) are expressed in total monthly amount received and temperature (°C) values are based on the monthly mean in 1999.

1.3.4 Overview

To address the questions proposed, this thesis is arranged into six chapters as outlined below:

Chapter 1

General introduction concerning the origin, morphology, annual life cycle and physiology of asparagus. Outlines the aims and overview of this study.

Chapter 2

In order to fully understand cultivar differences in photosynthesis in relation to spear yield under field-grown conditions, it was necessary to investigate seasonal and diurnal patterns of carbon assimilation. Measurements described in this chapter were an attempt to characterize the seasonal variations in photosynthetic parameters and causes of cultivar variation in photosynthetic capacity.

The contents of this chapter, in a modified form, have been accepted for publication in *Crop Science* (April, 2002, in press).

Chapter 3

The hypothesis that cultivar difference in spear yield was associated with assimilate partitioning by affecting carbohydrate buffer fluctuations in storage roots was tested

in this chapter. To do this, seasonal changes in carbohydrate partitioning and sucrose metabolism were determined. The role of source-sink interactions in the regulation of carbohydrate partitioning was also investigated.

The contents of this chapter, in a modified form, have been accepted for publication in *Functional Plant Biology* (May, 2002, in press).

Chapter 4

An important question which arises from chapter 2 and 3 relates to the carbon translocation that coordinates carbon assimilation and its partitioning into storage roots. A comparison of diel patterns in carbon assimilation, partitioning and export from mature cladophyll tissues between the two asparagus cultivars with contrasting yield was made to resolve the question: are rates of carbon export directly linked to the rates of carbon assimilation and sucrose concentration (feed-forward relationship) in mature cladophyll tissue?

The contents of this chapter, in a modified form, have been accepted for publication in *Physiologia Plantarum* (2002, in press).

Chapter 5

While there is a growing body of knowledge on the regulation of sucrose cleaving enzymes in carbon import into developing sinks, little is known of sucrose metabolism in relation to changes in physiological functions during tissue development. Previous studies in asparagus have illustrated a close correlation between fern vigour and spear yield, suggesting cultivar variations in activities of

sucrose-cleaving enzymes may exist. The aim of the experiments described in chapter 5 was to establish a relationship between spear extension and carbohydrate content as well as its metabolism in the developing spears.

The findings of this chapter have been published in *Australian Journal of Plant Physiology* (2001, Vol. 28:1013-1021).

Chapter 6

The final chapter consists of a general discussion and synthesis of the results presented in the body of this thesis. The implications of the findings to our understanding of carbon assimilation and partitioning as well as its utilization in relation to spear yield are discussed. Further questions are asked, along with suggestions for work which might follow.

CHAPTER 2

DIURNAL AND SEASONAL VARIATION IN PHOTOSYNTHESIS

Chapter 2

Diurnal and seasonal variation in photosynthesis

2.1 Introduction

Assimilate production is the most important physiological character underpinning the complex mechanisms which ultimately lead to crop yield (Stitt and Schulze 1994; Lawlor 1995). Although a positive relationship between photosynthetic rate and crop yield might be expected, findings of this nature are not common (Gifford and Evans 1981; Daie 1985; Evans 1993; Lawlor 1995). There are a number of likely reasons for this. Firstly, resources may be invested to maximise leaf area rather than directly invested in photosynthetic capacity. In this case, assimilate production at the whole plant level is increased, but not with an increase in the unit leaf area rate of photosynthesis (Stitt and Schulze 1994). Secondly, a portion of assimilates may be 'wasted', i.e. allocated to other organs and not contribute to crop yield (Pooter and Remkes 1990; Stitt and Schulze 1994). The link, therefore, between photosynthetic rate and crop yield is modified by strategies of assimilate partitioning and utilisation. Although the relationship between rate of CO_2 assimilation and crop yield has often been found to be decoupled, a positive correlation has been reported in a few cases (Zelitch, 1982; Peng *et al.*, 1991; Masle, 1992; Farquhar and Sharkey, 1994; Pettigrew and Meredith, 1994; Fischer *et al.*, 1998). Under some circumstance the

correlation between photosynthetic rate and dry matter production measured under field conditions at different times of the crop growth is indeed strong (Chandra Babu *et al.* 1985; Pooter and Remkes 1990; Pettigrew and Meredith 1994).

Asparagus, unlike most vegetable crops, is a perennial plant and current photosynthesis does not directly contribute to spear yield. Assimilate produced in summer is first translocated into storage roots and is subsequently utilised in vegetative growth during the next spring (Robb, 1984; Haynes, 1987; Pressman *et al.*, 1993). It is, therefore, expected that storage roots may act as a physiological buffer between carbon assimilation in the fern phase and its utilization in spear development. From a physiological point of view, the three stages of fern development (carbon assimilation), winter dormancy (carbon storage) and spear development (carbon utilization) provide a model system to investigate carbon assimilation, partitioning and its utilization. Recently, Faville *et al.* (1999b) reported a positive correlation between light saturated photosynthesis (A_{max}) of lateral ferns and spear yield among three asparagus cultivars. This finding was later confirmed by Bai and Kelly (1999) who found that spear yield and rate of canopy photosynthesis were significantly correlated among eight asparagus cultivars. These results suggest that genetic variation in photosynthetic capacity among asparagus cultivars may contribute to differences in spear yield.

In this chapter, the hypothesis that cultivar variations in spear yield in asparagus are associated with carbon assimilation was tested. One objective of this study was, therefore, to investigate diurnal and seasonal patterns of carbon assimilation under field conditions in two selected asparagus cultivars with significantly different yield. This is significant because the annual course of photosynthesis, and not the maximum rate, determines the annual plant carbon budget. The second aim of this study was to examine the relationship between photosynthetic capacity and parameters potentially limiting to photosynthesis. Specific attention was given to (1) effect of cladophyll age

on photosynthetic capacity; (2) the extent of stomatal and non-stomatal limitation to photosynthesis; (3) causes of cultivar variation in photosynthetic capacity.

2.2 Materials and methods

2.2.1 Microclimatic conditions

Site-specific changes of Photosynthetic photon flux density (PFD) measured at the top of the canopy during the measuring days were collected with a quantum sensor connected to the Li-Cor 6400 gas analysis system (Fig. 2.1).

2.2.2 Tissue age and harvesting

Cladophyll colour and size as well as sampling time were used as indication of age. Light green cladophylls approximately of 50% final size harvested in January were classified as expanding tissue. Green cladophylls of fully expanded size harvested in February were classified as fully expanded tissue. Dark green fully expanded cladophylls sampled in March were classified as mature tissue. Cladophylls with spot-yellow collected in April were classified as early senescent tissue, while the fully yellow cladophylls harvested in early May were classified as late senescent tissue. The samples destined for enzyme and protein determinations were quickly weighed and immediately frozen in liquid nitrogen and then stored at -80°C prior to assay.

Spear yield data were collected three days a week from the last week of October to the last week of November of 1998. At end of March 1999, measurements of plant height and shoot diameter were made.

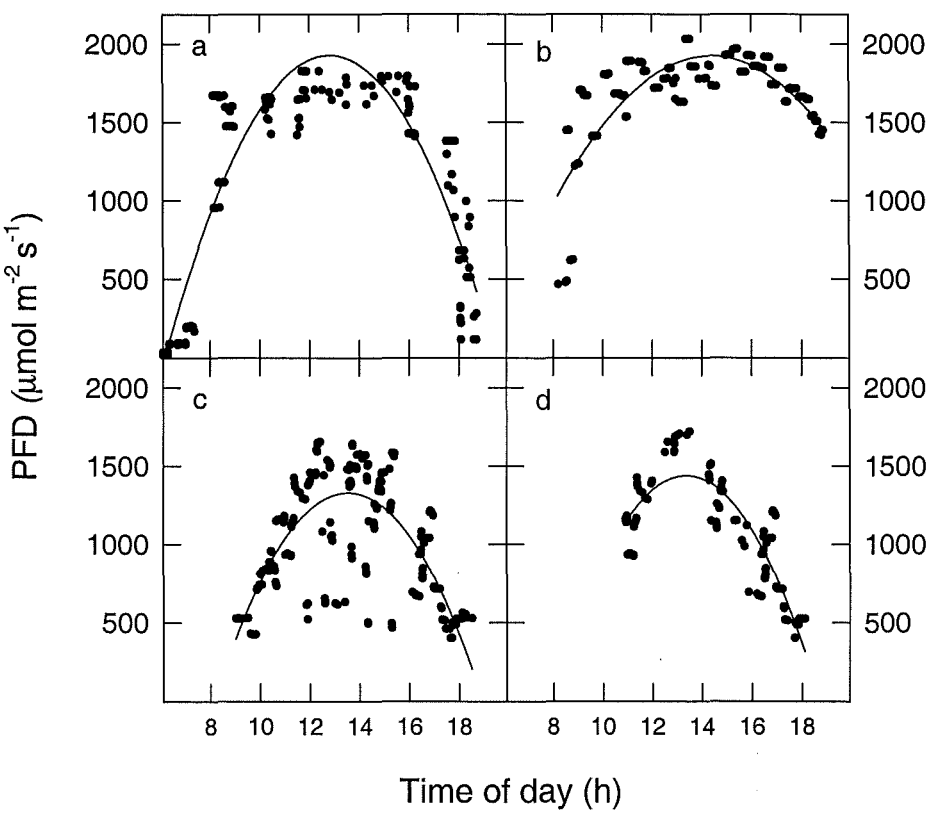


Fig. 2.1. Diurnal changes in photon flux density (PFD) during the photosynthesis measuring days (a = January; b = February; c = March; d = April) in the field. Each solid circle represents an individual record collected with a quantum sensor when the photosynthetic parameters were collected.

2.2.3 CO_2 assimilation measurements

The full diurnal courses of net photosynthetic rate (A) and stomata conductance (g_s) under ambient light conditions were measured monthly from January to April 1999 using a portable gas analysis system (Li-Cor model 6400, Lincoln, NE, USA) equipped with a CO_2 control module. All measurements were made on clear days except in March when measurements were made on a partially cloudy day. Each measurement was made by enclosing about 20-30 cladophylls (from the upper canopy receiving direct sunlight) in a clear-topped cuvette (2 × 3 cm) to make the total photosynthetic area approximately 4 cm^2. Cladophyll cuvette conditions during each measurement were maintained at 40% relative humidity, 25°C cuvette temperature and 35 Pa carbon dioxide concentration. Each determination was made when A had stabilized — this process typically took 1-2 min. After each measurement, cladophylls used for the A measurement were collected and their total surface area was calculated according to the equation:

$$\text{Area} = \sum_{n} (L \times D \times \pi)$$

Where D is cladophyll diameter and L is the length of the cladophyll and n is the number of cladophylls used.

The responses of A to intercellular CO_2 partial pressure (A/C_i response) and to irradiance (A/PFD response) were determined on different clear days with similar conditions between 1030 h to 1500 h. An umbrella was used to shade the Li-Cor during the measurements to avoid overheating. For A/C_i response determination, external CO_2 concentrations (C_a) were supplied in 10 steps from 5 to 80 Pa. Measurements were made at each C_a point when gas exchange had equilibrated, at

which point, the coefficient of variation (CV) for the CO_2 concentration differential between the sample and reference analysers was below 1%. Cladophyll temperature was maintained at 25°C using thermoelectric coolers, and water vapour pressure deficit (VPD) was generally held between 1.2 and 1.7 kPa. A constant PFD of 2000 μmol m^{-2} s^{-1} was provided by blue-red light-emitting diodes mounted above the chamber.

The response of A to PFD was determined in the cuvette at PFDs of 2000, 1500, 1000, 800, 500, 300, 200, 150, 100, 75, 50, 25 and 0 μmol m^{-2} s^{-1}. The carbon dioxide concentration in the cuvette was held at 35 Pa. Other conditions in the cuvette were similar to those in A/C_i curve determinations. Dark respiration (R_d) was measured directly from light response at zero irradiance. Values of light saturated photosynthesis (A_{max}) were estimated using Photosynthesis Assistant (Dundee Scientific, UK) which uses a quadratic equation established by Prioul and Chartier (1977).

Analysis of A/C_i responses involved calculation of parameters potentially limiting to photosynthesis: V_{cmax} (maximum carboxylation rate of rubisco) and J_{max} (RuBP regeneration capacity mediated by maximum electron transport rate). This was achieved using Photosynthesis Assistant (Dundee Scientific, UK) which uses the biochemical model describing A by Farquhar *et al.* (1980):

$$A = \min \{A_c, A_q\} - R_d$$

Where A_c and A_q are the assimilation rates limited by rubisco activity and electron transport rate respectively, and min {} refers to the minimum of the two rates. R_d is daytime respiration rate resulting from processes other than photorespiration. When photosynthesis is limited by rubisco activity, A_c is given by

$$A_c = V_{cmax}\frac{C_i - \Gamma *}{C_i + K_c[1 + (\frac{O_i}{K_o})]}$$

Where Γ^* is the CO_2 compensation point in the absence of day respiration, K_c and K_o are Michaelis constants for CO_2 and O_2, respectively, and O_i is the intercellular O_2 concentration. When CO_2 assimilation is limited by regeneration of RuBP via electron transport, A_q is described as

$$A_q = \frac{J(C_i - \Gamma^*)}{4(C_i + 2\Gamma^*)}$$

Where J is the electron transport rate for a given quantum irradiance. Comparison of photosynthetic characteristics between the two cultivars was assisted by calculation of A_{sat} (net photosynthetic rate at a CO_2 concentration of 80 Pa and saturating PFD, 2000 μmol m^{-2} s^{-1}), C_i/C_a (the ratio of internal to external CO_2 partial pressure under saturating PFD at ambient C_a) and the relative stomatal limitation calculated according to the equation

$$L_{stom} = 1 - \frac{A}{A_o} \times 100$$

Where A is the net assimilation rate at the growth C_a (35 Pa), and A_o is the net photosynthetic rate at a C_a resulting in a C_i equal to the growth C_a (Farquhar and Sharkey 1982).

2.2.4 Rubisco activity

Fresh cladophyll tissue (0.5 g) was collected from the field and immediately frozen in liquid nitrogen. Rubisco enzyme was extracted by grinding frozen tissue in a chilled mortar and pestle with 5 mL of HEPES-KOH extraction buffer (pH 7.5), containing 20 m*M* $MgCl_2$, 50 m*M* HEPES, 5 m*M* EDTA, 2% (w/v) polyvinyl-polypyrolidone (PVP), 15% (w/v) PEG, 14 m*M* B-mercaptoethanol and 1% (v/v) Tween 80. Immediately after extraction, the extract was centrifuged for 3 min at 15,000 × g and 4°C, and the supernatant was then assayed for rubisco activity. Determination of rubisco activity was performed according to Tissue *et al.* (1993). The initial (*in vivo*) and total (fully activated) activities of rubisco were analysed spectrophotometrically in a coupled assay which measures the rate of disappearance of the reduced form of nicotinamide adenine dinucleotide (NADH). For each sample, initial activity was determined immediately after extraction and total activity was determined 15 min after fully carbamylating the enzyme. The initial activity was assayed at 25°C for 2 min by adding 70 μL of sample extract into 100 m*M* bicine-KOH (pH 8) containing 25 m*M* $KHCO_3$, 20 m*M* $MgCl_2$, 3.5 m*M* ATP, 5 m*M* phosphocreatine, 80 nkat of glyceraldehyde-3-phosphate dehydrogenase (Sigma G-8380), 80 nkat of 3-phosphoglyceric phosphokinase (Sigma P-1136), 80 nkat of creatine phosphokinase (Sigma C-3755), 0.25 m*M* NADH and 0.5 m*M* ribulose-1,5-bisphosphate (RuBP). In the assay of total rubisco activity, 70 μL of extract was injected into the above assay buffer without RuBP and then incubated at 25°C for 15 min. Activity was then measured by adding RuBP. Enzyme activation state was calculated from the ratio of the initial to total rubisco activities.

Chlorophyll fluorescence

Chlorophyll fluorescence of cladophylls measured with a portable fluorometer (Mini-PAM-2000, Walz, Germany) was used to assess the photochemical efficiency of photosystem II (PSII). Intact cladophylls were dark adapted for 30 min using a dark clip holder. Minimal fluorescence F_o of the dark-adapted cladophylls was determined by exciting the cladophylls with weak modulated radiation (LED 655 nm) of 0.15 μmol m^{-2} s^{-1} at frequency of 0.6 kHz. Thereafter, a saturating pulse of 4500 μmol m^{-2} s^{-1} was applied through a fibre optic cable for 400 ms to obtain maximum fluorescence F_m. Maximum photochemical efficiency of PSII (F_v/F_m) was calculated using the formula $(F_m-F_o)/F_m$ (Bolhàr-Nordenkampf and Öquist 1993).

2.2.5 Additional measurements

Total soluble protein was extracted from cladophyll tissue with the same buffer used in rubisco extraction by grinding approximately 0.5 g of fresh sample in a pre-cooled mortar with 5 mL buffer and then centrifuging for 3 min at 15,000 × g and 4°C. The resulting supernatant was assayed for soluble protein by the method of Bradford (1976) using BSA as a standard protein. Chlorophyll content was determined according to Sestak (1971) by grinding about 0.3 g fresh tissue in liquid nitrogen, double extracting with 2 mL of 80% (v/v) acetone, centrifuging for 5 min at 11,000 × g and 4°C, and measuring absorbance of the supernatant at 647 and 664 nm. Shoot xylem water potential (Ψ) was measured on second shoot terminal branches at midday with a pressure chamber (PMS Instrument CO, Corvallis, Oregon, USA). Shoot relative water content (RWC) was determined in separate branches and calculated by

$$RWC = \frac{FW + DW}{FW_{sat} + DW} \times 100\%$$

where FW is fresh weight of tissue, FW_{sat} is the water saturated weight after absorbing water over night and DW is the dry weight after 48 h at 70°C. Freeze-dried cladophylls were used for soluble sugar and starch determination using a phenol-sulfuric acid method (Tissue and Wright 1995).

2.2.6 Statistical analysis

One way analysis of variance (ANOVA) was used to test for difference between the two cultivars. Effect of cladophyll age on photosynthetic parameters was analyzed using Tukey's test. Differences were considered significant if $P < 0.05$.

2.3 Results

2.3.1 Plant growth analysis

Plant growth data are given in Table 2.1. Spear harvest in the previous season revealed a significantly greater yield in ASP-69 than in ASP-03. ASP-69 also displayed greater final shoot height and diameter in comparison to ASP-03.

	Spear yield g FW/plot	Plant height (cm)	Shoot diameter (mm)
ASP-69	3587 ± 551	175 ± 5	12.2 ± 0.6
ASP-03	974 ± 90	120 ± 5	8.9 ± 0.6
ANOVA	*	***	***

Table 2.1. Spear yield, plant height and shoot diameter of two asparagus cultivars grown in the field. Results are means ± SE of 3 plots for spear yield and 12 replicates for shoot height and diameter. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated as: * $P < 0.05$, *** $P < 0.001$.

2.3.2 Diurnal and seasonal changes in A and g_s

In both cultivars investigated, diurnal patterns of *A* generally paralleled changes in PFD and exhibited a sinusoidal pattern (Fig. 2.2). In the rapidly expanding cladophyll tissue measured in January, the two cultivars exhibited similar values of *A* throughout the day, whereas in the fully expanded cladophyll tissue measured in February, a significant difference in *A* between the two cultivars was observed throughout the measuring period. Measurements of *A* in mature cladophyll tissues (March) showed reduction in *A* in both cultivars in comparison to fully expanded cladophyll tissue. However, a consistent difference between the two cultivars was still evident, particularly in the morning. The diurnal patterns of *A* in senescent cladophyll tissue measured in April were clearly different from the actively photosynthesizing cladophyll tissue in February. After reaching a peak at approximately 1300 h, both cultivars displayed a consistent decline until the end of the day.

Variation in *A* not only differed with time of day but also changed throughout the growing season in the two cultivars (Fig. 2.2). Photosynthetic rate reached a peak in fully expanded tissues (February) of 8.94 ± 0.54 μmol m^{-2} s^{-1} in ASP-69 and 6.50 ± 0.38 μmol m^{-2} s^{-1} in ASP-03, and then declined until the end of the fern growing season. The time at which maximum rate of photosynthesis was observed shifted from mid-morning in January to early afternoon in April. The values of *A* measured in senescent cladophyll tissue in April were characterised by a significant decline in both cultivars although PFDs were still above those capable of saturating photosynthesis.

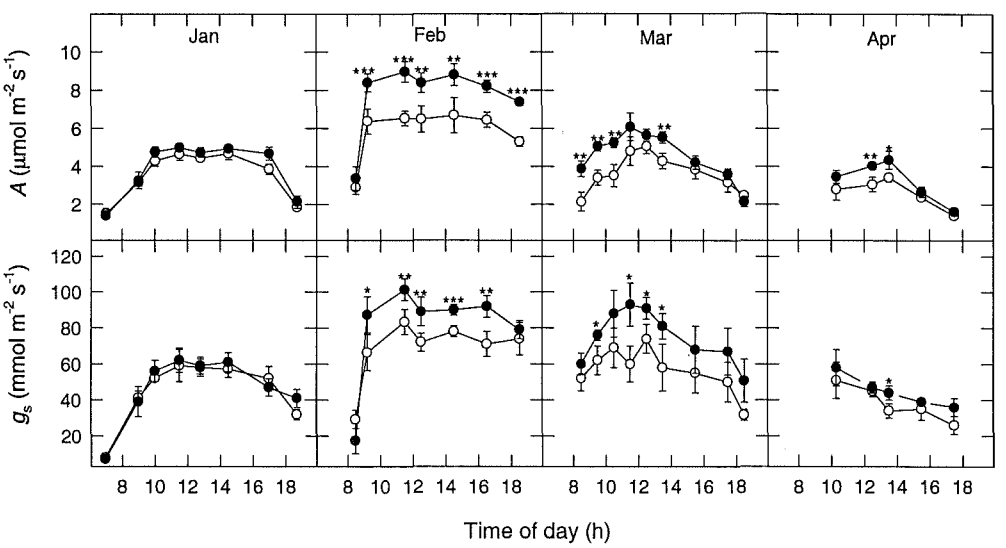


Fig. 2.2. Diurnal and seasonal changes in the rate of net photosynthesis (*A*) and stomatal conductance (g_s) between ASP-69 (closed symbol) and ASP-03 (open symbol). Results are means of 6 replicates ± SE. Significant differences between ASP-69 and ASP-03 are indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Diurnal courses in g_s generally paralleled those in A in both cultivars (Fig. 2.2). In the rapidly expanding cladophyll tissue (January), values of g_s were similar for the two cultivars. In the fully expanded cladophyll tissue (February), there was a significant difference in g_s between the two cultivars throughout the day. Maximum g_s was 104 ± 6 mmol m^{-2} s^{-1} for ASP-69 and 83 ± 7 mmol m^{-2} s^{-1} for ASP-03, respectively. A similar trend was observed in mature cladophyll tissue measured in March, but g_s was lower. In senescent cladophyll tissue (April), there was less difference between the two cultivars, although g_s in ASP-69 was slightly higher than in ASP-03. A close correlation between maximum g_s and A was found in both cultivars (r = 0.84; Fig. 2.3).

2.3.3 A/C_i and A/PFD responses and cladophyll fluorescence characteristics

Analysis of the fitted curves of A/C_i relationships allowed determination of V_{cmax}, J_{max}, A_{sat} and the limitation imposed by the stomata (L_{stom}) on the rate of photosynthesis (Table 2.2). The response of photosynthesis to C_i in fully expanded and mature cladophylls differed between cultivars, with significant cultivar difference in values of V_{cmax} and A_{sat} observed. ASP-69 had significantly higher values in A_{sat} than in ASP-03 at all stages of development, whereas values of V_{cmax} in ASP-69 were significantly greater than in ASP-03 in fully expanded and mature cladophylls. A significant reduction in photosynthetic capacity was observed in senescent cladophylls (April). This reduction was reflected in A_{sat} values which were reduced by 30% in ASP-03 and 35% in ASP-69 in comparison to those in February. Similarly, both V_{cmax} and J_{max} were also significantly reduced in senescent cladophylls. L_{stom} was high in all aged cladophylls (> 40%) in both cultivars except in the fully expanded cladophyll. The C_i/C_a ratio, which reflects changes in the relationship between stomatal conductance and biochemical capacity for photosynthesis, was not affected by cladophyll age. Both L_{stom} and C_i/C_a values did not differ significantly between the two cultivars.

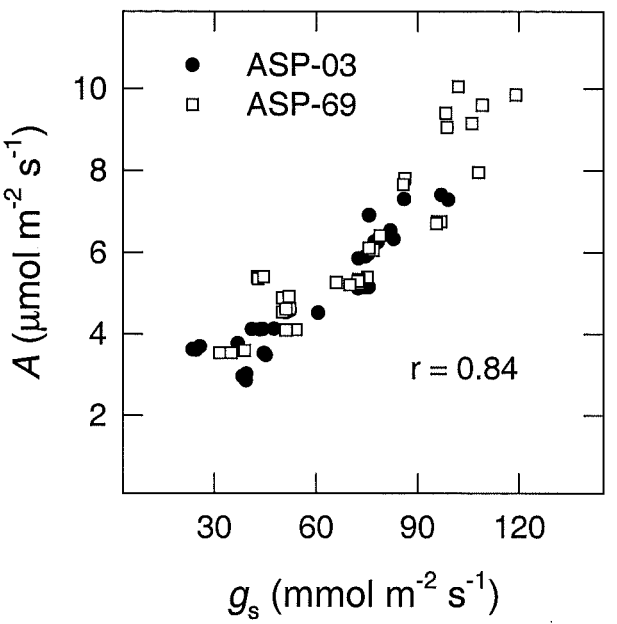


Fig. 2.3. Relationship between photosynthetic rate and stomatal conductance by plotting individual values of maximum net photosynthetic rate from each measuring days against concurrent stomatal conductance values.

Parameter		February (fully expanded)	March (mature)	April (senescent)
V_{cmax}	ASP-03	25.25 ± 0.71^{a}	27.31 ± 1.19^{a}	21.43 ± 0.80^{b}
(μmol m^{-2} s^{-1})	ASP-69	31.47 ± 1.93^{a}	31.30 ± 1.49^{a}	25.24 ± 2.33^{a}
	ANOVA	*	*	ns
J_{max}	ASP-03	62.0 ± 0.4^{a}	62.9 ± 2.2^{a}	51.1 ± 2.3^{b}
(μmol m^{-2} s^{-1})	ASP-69	73.2 ± 1.8^{a}	70.9 ± 3.6^{ab}	54.7 ± 4.7^{b}
	ANOVA	ns	ns	ns
A_{sat}	ASP-03	9.15 ± 0.09^{a}	8.31 ± 0.22^{b}	5.72 ± 0.19^{c}
(μmol m^{-2} s^{-1})	ASP-69	10.81 ± 0.31^{a}	10.21 ± 0.40^{a}	6.59 ± 0.31^{b}
	ANOVA	**	**	*
L_{stom}	ASP-03	39.95 ± 5.79^{a}	56.98 ± 5.15^{ab}	48.87 ± 1.74^{b}
(%)	ASP-69	32.55 ± 4.80^{a}	56.17 ± 0.22^{b}	43.30 ± 3.39^{c}
	ANOVA	ns	ns	ns
C_i/C_a	ASP-03	0.62 ± 0.05^{a}	0.52 ± 0.05^{a}	0.58 ± 0.02^{a}
(ratio)	ASP-69	0.64 ± 0.03^{a}	0.52 ± 0.02^{a}	0.61 ± 0.01^{a}
	ANOVA	ns	ns	ns

Table 2.2. Parameters derived from A/C_i relationships in fully expanded (February), mature (March) and senescent (April) cladophylls: maximum rate of carboxylation (V_{cmax}), the potential rate of RuBP regeneration (J_{max}), net assimilation rate at saturating CO_2 concentration of 80 Pa and saturating PFD (A_{sat}), relative stomatal limitation (L_{stom}) and ratio of intercellular to atmospheric CO_2 (C_i/C_a). Mean values (4 replicates) ± SE are compared using one way ANOVA (ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Different letters within rows indicate statistically different values $P < 0.05$ using Tukey's test.

The response of A to PFD was analysed in fully expanded, mature and senescent cladophylls (Fig. 2.4 and Table 2.3). The two cultivars exhibited similar patterns of response with age, with a decrease in photosynthetic capacity evident as the season progressed. However, the significant decline in light saturated photosynthesis (A_{max}) with age was only observed in senescent cladophyll tissue. Comparison between the two cultivars indicated that ASP-69 had significantly higher values of A_{max} than those of ASP-03 in all age cladophylls. There was no significant difference in dark respiration (R_d) between the two cultivars. In both cultivars, photochemical efficiency (F_v/F_m) was constant until the late senescent stage measured in May (Fig. 2.5a). There was no significant difference in F_v/F_m between the two cultivars. Both cultivars showed a noon reduction in F_v/F_m, although this reduction was rather small and was reversed by late afternoon except in senescent cladophylls (data not shown).

2.3.4 Rubisco activity

The seasonal courses of initial and total rubisco activity displayed similar patterns in the two cultivars and generally paralleled changes in photosynthetic rate throughout cladophyll development. As cladophyll tissue expanded, both initial and total rubisco activity increased, reaching a maximum in mature cladophyll tissue and then declining following the onset of senescence (Table 2.4). Initial rubisco activity, which reflects *in vivo* activity, was significantly higher in ASP-69 than in ASP-03 ($P < 0.01$) except in expanding cladophyll tissue. Similar trends were also observed in total rubisco activity. The activation state of rubisco enzyme did not differ between the two cultivars. Diurnal changes in initial and total rubisco activity were measured in mature cladophyll tissue in March (Table 2.5). In both cultivars, the initial and total rubisco activity was slightly lower in the morning and slightly higher at both noon and end of the day. A significantly greater initial and total rubisco activity in ASP-69 than in ASP-03 was found consistently throughout the day. However, a significant correlation between rubisco activity and photosynthetic rate did not exist.

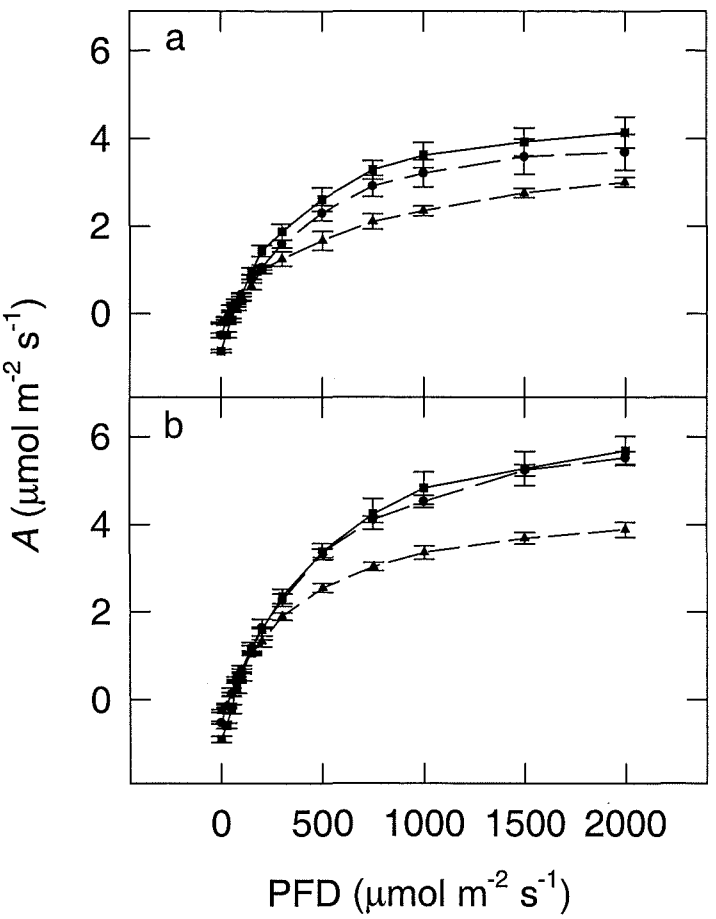


Fig. 2.4. The relationship between irradiance (PFD) and photosynthetic rate (*A*) in fully expanded (▪), mature (•) and senescent cladophylls (▲) for the two asparagus cultivars (a, ASP-03; b, ASP-69) grown in the field. Results are means of 4 replicates ± SE.

Parameter		February (fully expanded)	March (mature)	April (senescent)
R_d	ASP-03	0.85 ± 0.07^a	0.44 ± 0.05^b	0.22 ± 0.04^c
(μmol m^{-2} s^{-1})	ASP-69	0.94 ± 0.06^a	0.49 ± 0.03^b	0.32 ± 0.05^c
	ANOVA	ns	ns	ns
A_{max}	ASP-03	5.69 ± 0.46^a	4.77 ± 0.55^{ab}	3.89 ± 0.35^b
(μmol m^{-2} s^{-1})	ASP-69	7.82 ± 0.52^a	7.24 ± 0.20^a	4.80 ± 0.31^b
	ANOVA	***	***	*

Table 2.3. Parameters derived from response of CO_2 assimilation rate to irradiance (PFD) in fully expanded, mature and senescent cladophylls. Mean values (4 replicates) ± SE are compared using one way ANOVA (ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Different letters within rows indicate statistically different values $P < 0.05$ using Tukey's test.

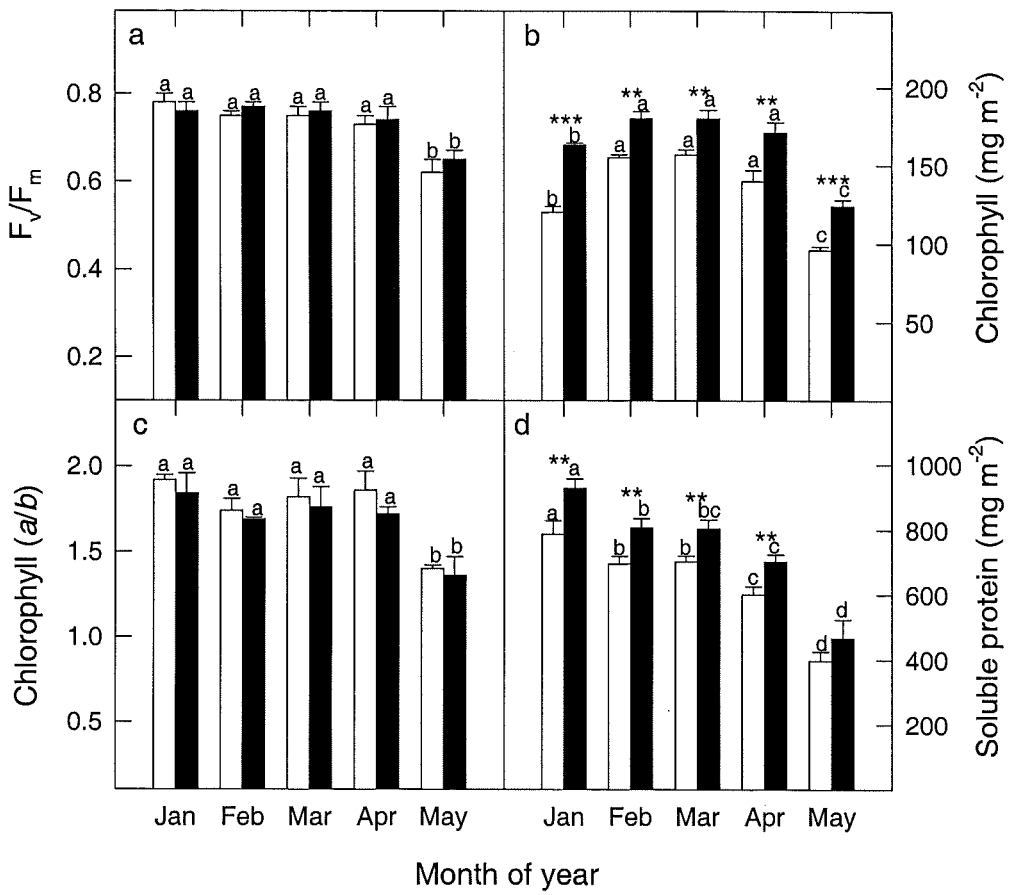


Fig. 2.5. Seasonal changes in maximum photochemical efficiency F_v/F_m (*a*), total chlorophyll content (*b*), chlorophyll *a*/*b* ratio (*c*) and soluble protein content (*d*) measured between 1100 to 1200 h in cladophylls of ASP-69 (closed bar) and ASP-03 (open bar). Results are means of 6 replicates ± SE. Significant differences between ASP-69 and ASP-03 are indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Different letters within cultivars indicate statistically different values $P < 0.05$ using Tukey's test.

Parameter		January (expanding)	February (fully expanded)	March (mature)	April (senescent)
Initial activity	ASP-03	1.08 ± 0.14^{a}	1.98 ± 0.36^{b}	2.17 ± 0.25^{b}	1.63 ± 0.22^{ab}
(μmol m^{-2} s^{-1})	ASP-69	1.94 ± 0.53^{a}	3.60 ± 0.41^{b}	3.63 ± 0.47^{b}	2.44 ± 0.21^{a}
	ANOVA	ns	**	**	**
Total activity	ASP-03	1.96 ± 0.14^{a}	3.02 ± 0.43^{ab}	4.55 ± 0.48^{c}	2.31 ± 0.32^{ab}
(μmol m^{-2} s^{-1})	ASP-69	2.97 ± 0.65^{a}	5.18 ± 0.49^{b}	6.72 ± 0.47^{c}	3.21 ± 0.19^{a}
	ANOVA	ns	***	***	**
Activation state	ASP-03	55 ± 5.5^{ac}	65 ± 4.9^{ab}	48 ± 4.2^{c}	72 ± 8.2^{b}
(%)	ASP-69	64 ± 5.9^{ab}	70 ± 4.9^{ab}	54 ± 4.1^{a}	76 ± 8.0^{b}
	ANOVA	ns	ns	ns	ns

Table 2.4. Initial (*in vivo*) and total (fully activated) activities and activation state of rubisco enzyme. All samples were collected between 1030 to 1130 h on the measuring days. Mean values (3 replicates) ± SE are compared using one way ANOVA (ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Different letters within rows indicate the significance of age effect at $P < 0.05$ using Tukey's test.

Parameter		Time of day 9:00	12:00	20:00
Initial activity	ASP-03	1.95 ± 0.18	2.17 ± 0.25	2.21 ± 0.38
(μmol m^{-2} s^{-1})	ASP-69	3.02 ± 0.23	3.63 ± 0.47	3.72 ± 0.29
	ANOVA	**	***	*
Total activity	ASP-03	3.90 ± 0.47	4.55 ± 0.48	4.14 ± 0.67
(μmol m^{-2} s^{-1})	ASP-69	6.17 ± 0.75	6.72 ± 0.47	6.46 ± 0.51
	ANOVA	***	**	*
Activation state	ASP-03	51 ± 5.6	48 ± 4.2	55 ±11.0
(%)	ASP-69	50 ± 5.3	54 ± 4.1	58 ± 6.1
	ANOVA	ns	ns	ns

Table 2.5. Changes in rubisco activity during the day in mature cladophyll tissue (measured in March) of two asparagus cultivars grown in the field. Results are measurements of six replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated as: ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

2.3.5 Cladophyll properties

Chlorophyll content increased initially with age in both cultivars, reaching a maximum of 180 ± 5 mg m^{-2} in ASP-69 and 157 ± 3 mg m^{-2} in ASP-03 in mature cladophylls measured in March, and then declined in senescent cladophylls (Fig. 2.5b). ASP-69 possessed significantly greater chlorophyll content than ASP-03 on an area basis throughout the season. However, chlorophyll *a/b* ratio did not differ significantly between the two cultivars (Fig. 2.5c). A significant decrease in chlorophyll *a/b* ratio occurred in the late senescent cladophylls (May). In both cultivars, the highest total soluble protein content was found in rapidly expanding cladophyll tissue and declined as the season progressed (Fig. 2.5d). ASP-69 had a significantly greater soluble protein content than in ASP-03 on an area basis over the season. Midday shoot xylem water potential (Ψ) decreased initially and reached a minimum of -2.20 ± 0.18 MPa in ASP-69 and -2.24 ± 0.14 MPa in ASP-03 in February after which it increased towards the end of the fern growing season. There was no significant difference between cultivars (Fig. 2.6). Relative water content (RWC) also did not differ significantly between the two cultivars (Fig. 2.6).

Cladophyll diameter in ASP-69 was significantly greater than in ASP-03 (Table 2.6). The greater size in ASP-69 was associated with a greater specific leaf weight (SLW) of 2.47 ± 0.03 mg cm^{-2} in comparison to ASP-03 with 2.18 ± 0.05 mg cm^{-2}. Soluble sugar content was greater in ASP-69 than in ASP-03, whereas starch content did not differ significantly between the two cultivars in mature cladophylls (Table 2.6).

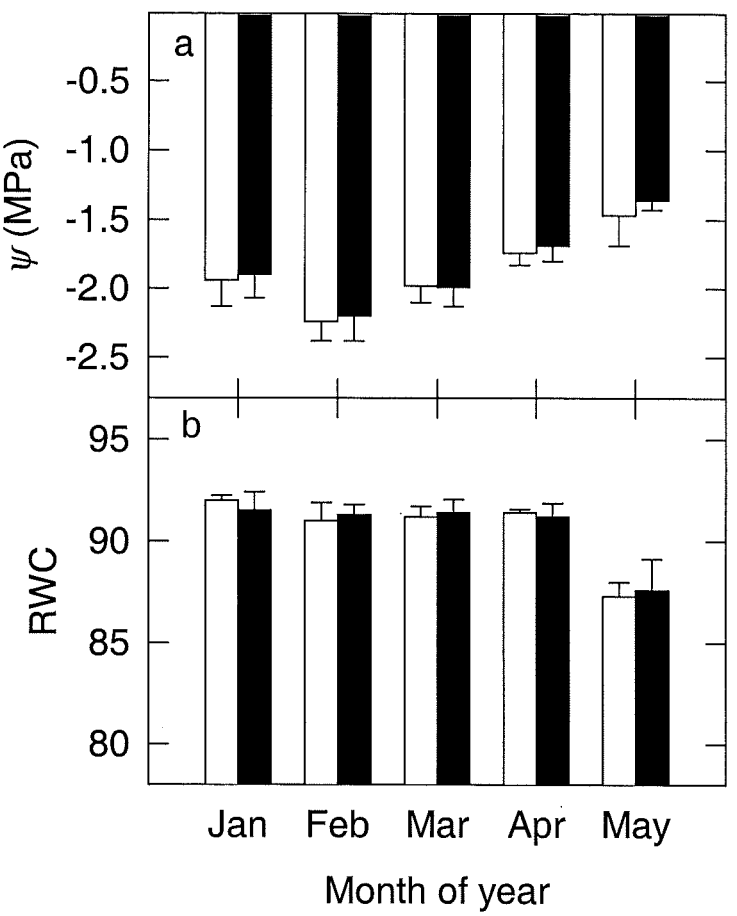


Fig. 2.6. Seasonal changes in shoot xylem water potential (Ψ) and relative water content (RWC) in cladophylls of ASP-69 (closed bar) and ASP-03 (open bar) measured in the middle of day. Results are means of 3 replicates ± SE.

Parameter	Cladophyll diameter (mm)	SLW (mg cm^{-2})	Soluble sugar (mg cm^{-2})	Starch (mg cm^{-2})
ASP-03	0.36 ± 0.011	2.18 ± 0.047	0.36 ± 0.027	0.12 ± 0.007
ASP-69	0.39 ± 0.013	2.47 ± 0.033	0.41 ± 0.020	0.13 ± 0.010
ANOVA	***	***	*	ns

Table 2.6. Comparison of cladophyll diameter, specific leaf weight (SLW) and carbohydrate content (soluble sugar and starch) in mature cladophylls of ASP-03 and ASP-69. All values were determined between 1130 to 1430 h in mid-summer of 1999. Results for cladophyll diameter and SLW are means of 12 replicates ± SE. Results for soluble sugar and starch are 6 replicates ± SE. Mean values are compared using one way ANOVA (ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

2.4 Discussion

There has been considerable interest in the physiological characters (eg. respiration, photosynthetic rate, stomatal conductance and rubisco activity) underpinning genotypic differences in plant yield (Evans 1994; Stitt and Schulze 1994; Lawlor 1995). Although such characters have often proved to vary in their effect on plant yield, a positive correlation between carbon assimilation and crop yield has been reported in some species (Peng *et al.* 1991; Masle 1992; Farquhar and Sharkey 1994; Bai and Kelly 1999; Faville *et al.* 1999b). The results obtained in this study are consistent with the finding of Faville *et al.* (1999) that A_{max} is positively associated with spear yield. In addition, the high-yielding cultivar ASP-69 displays significantly greater V_{cmax}, rubisco activity, g_s, SLW and cladophyll diameter in comparison to the low-yielding cultivar ASP-03. These results strongly suggest that differences in both biochemical and leaf structural characteristics influence photosynthetic capacity and may contribute to yield differences in the two cultivars studied.

2.4.1 Effect of cladophyll age on photosynthetic parameters

In temperate conditions, photosynthetic activity in asparagus is maintained for approximately four months from late spring to late summer after which all cladophyll tissue senesces within a few weeks in autumn. Given such a short growth period, it is crucial for the plants to reach maximum photosynthetic capacity quickly and to maintain photosynthesis for as long as possible. The results obtained from this study indicate that both cultivars had high rates of A in rapidly expanding cladophyll tissue and attained maximum rates of net photosynthesis in fully expanded cladophyll tissue when seasonal radiation and temperature were both at a maximum. These results are in agreement with those of Downton and Törökfalvy (1975) who found that net photosynthesis occurs once the cladophyll begins to assume a needle-like form.

While photosynthetic rate reached a maximum in fully expanded tissue, rubisco activity did not reach a maximum until March in mature cladophylls, and the correlation between photosynthetic rate and rubisco activity did not exist. This discrepancy may be a result of higher activation states of rubisco in the fully expanded cladophyll tissue compensating for low rubisco content. In some species, like willow (*Salix cv. Aquatica gigantea*), activation state has been found to be higher early in the season and to decrease gradually as leaves aged (Vapaavuori and Vuorinen 1989). Although rubisco content was not measured in this study, total rubisco activity may be taken as an indicator of the quantity of this enzyme (Gazelius and Widell 1986; Merlo *et al.* 1992). Thus, rubisco content in mature cladophyll tissue is likely to be higher than in fully expanded tissue in both cultivars. Nevertheless, both initial and total rubisco activities in ASP-69 were significantly greater than in ASP-03, indicating the important role of rubisco enzyme to cultivar differences in photosynthetic capacity.

Rubisco activities measured in this study appear lower than would be expected based on previous studies. In general, total rubisco activity is considered to approximate values for V_{cmax} calculated from A/C_i curves (Farquhar *et al.* 1980; Farquhar and Sharkey 1982). Effort was made to modify extraction protocols to ensure that the majority of rubisco protein was extracted in an active state, but without significant success. Rubisco data are still presented here as they show a consistent cultivar difference in activity. Clearly, further work is needed to improve rubisco extraction and assay in asparagus.

Both cultivars showed a significant decline in A in senescent cladophyll tissue in April, although most of the senescing cladophylls retained their chlorophyll content. The decline in A was accompanied by reduced stomatal conductance and rubisco activity and also by shorter day lengths and colder temperature at night. Although rubisco activity declined significantly, the activation state of rubisco remained unchanged. The activation state of rubisco is thought to be influenced primarily by the

action of the ATP-dependent rubisco activase and ATP supply, which in turn is limited by electron transport rate (Kobza and Seemann 1989). The lack of a significant decrease in F_v/F_m in senescent cladophyll tissue in April in comparison to May indicates that photochemical capacity was maintained and implies that activation state of rubisco was not necessarily limited by the availability of ATP. Non-stomatal limitations on photosynthesis at this time were probably due to the biochemical inactivation of photosynthetic enzymes. In the senescent cladophyll tissue measured in May, the decrease in photosynthesis was accompanied by a significant decline both in F_v/F_m and chlorophyll content, indicating a loss of both reaction centers and light harvesting complexes of PSII (Massacci and Jones 1990). Furthermore, the rapid decrease in the chlorophyll *a/b* ratio in May indicates that the PSII reaction center complexes, which contain only chlorophyll *a*, were degraded more than the light-harvesting chlorophyll-protein (Peñarrubia and Morebo 1995). These results suggested that cladophyll senescence has two phases. In the first phase, the decrease in *A* may be explained by the existence of an increased mesophyll resistance due to biochemical inactivation of photosynthetic enzymes or decreased enzyme pools caused by an extended cold period (Hansen *et al.* 1996; Schwarz *et al.* 1997). In the second phase, there is an overall reduction both in reaction centers and light harvest complexes of PSII (Peñarrubia and Morebo 1995).

In the current study, mid-day depression of photosynthesis was not observed in either of the two cultivars even though PFD exceeded 1500 $\mu mol\ m^{-2}\ s^{-1}$ at noon on all the measuring days. Rates of photosynthesis were well maintained and generally aligned with diurnal changes in PFD. Neither cultivar displayed a significant decline in F_v/F_m during the days measured. F_v/F_m always recovered to maximum values at night except in senescing cladophylls. This suggests that both cultivars are fully adapted to present environmental conditions and do not suffer photoinhibition.

2.4.2 Stomatal and non-stomatal limitation to photosynthesis

Both stomatal and non-stomatal (metabolic) factors have been shown to limit photosynthetic capacity, and the impact of the two factors varies among species or genotypes in response to environmental conditions (Hutmacher and Krieg 1983; Briggs *et al.* 1986; Teskey *et al.* 1986). Under dry soil conditions, it is generally believed that stomatal limitation to CO_2 influx into the mesophyll is the primary cause of the photosynthetic depression observed in mature tissue (Quick *et al.* 1992; Kicheva *et al.* 1994; Ishida *et al.* 1999). However, relative reductions in photosynthesis compared to stomatal conductance have been observed in several species under dry soil conditions (Ni and Pallardy 1992). In addition, decreased stomatal conductance may be the result rather than the cause of decreased photosynthesis (Fiscus *et al.* 1997). Under other circumstances (elevated CO_2), metabolic limitation at the chloroplast level may exercise a greater degree of control over photosynthesis than stomatal conductance (Noormets *et al.* 2001).

In the present study, both cultivars had significantly lower net photosynthesis rates in the senescent cladophyll measured in April in comparison to the mature cladophyll measured in March. The decline in A was accompanied by a decrease in stomatal conductance and rubisco activity, whereas C_i/C_a ratio, which directly reflects changes in the relationship between stomatal conductance and the biochemical capacity for CO_2 fixation (Tissue *et al.* 1995), was similar in these two cultivars. Relative stomatal limitation to photosynthesis (L_{stom}) was also not significantly different between the two cultivars. These results indicate a close coordination between stomatal conductance and biochemical capacity for CO_2 assimilation in the two cultivars. However, the decline in A without a corresponding decline in C_i/C_a in the early senescent cladophyll suggests that the stomatal response was secondary to changes in mesophyll processes (Noormets *et al.* 2001).

2.4.3 Causes of cultivar variation in photosynthetic rate

Parameters derived from A/C_i and A/PFD relationships and the rubisco assay provide some understanding as to the causes for the different photosynthetic capacities between the two asparagus cultivars. Values of A_{sat}, A_{max} and V_{cmax} in ASP-69 were significantly greater than in ASP-03 and greater net photosynthesis in ASP-69 was always associated with greater rubisco activity in comparison to ASP-03. Apart from the physiological differences, differences in cladophyll properties are also likely to play a significant role in determining the rate of photosynthesis in the two cultivars. The rate of photosynthesis can be affected by various anatomical features of the leaves, including mesophyll size and arrangement. In this study, significant difference existed in cladophyll thickness. The high-yielding cultivar (ASP-69) had thicker cladophylls and greater specific leaf weight (SLW) than in ASP-03. Similar results have also been reported by Faville *et al.* (1999) and Bai and Kelly (1999). Obviously, an increase in thickness will influence mesophyll volume for a given surface area. The present observations are thus consistent with the idea that the greater biochemical activity in ASP-69 is partly due to greater photosynthetic machinery resulting from thicker cladophyll tissue. The close relationship between cladophyll size and photosynthetic capacity among asparagus cultivars raises the possibility of selecting high-yielding cultivars with greater photosynthetic rate without significantly reducing canopy size. This approach needs further investigation. The overall data presented here highlight the conclusion that apart from significant differences in canopy size, photosynthetic capacity per unit leaf area is also a significant factor contributing to differences in assimilate production between the two cultivars, and both physiological and anatomical factors appear to play significant roles in determining differences in photosynthetic capacity.

2.5 Summary

In order to investigate physiological characters underpinning spear yield in asparagus (*Asparagus officinalis* L.), diurnal and seasonal changes in photosynthetic parameters were measured under field conditions in two cultivars with contrasting yield. Seasonal patterns in photosynthetic parameters were strongly dependent on cladophyll developmental stage in both cultivars. The greatest photosynthetic rates (A) of 8.94 ± 0.54 μmol m^{-2} s^{-1} for the high-yielding cultivar (ASP-69) and 6.50 ± 0.38 μmol m^{-2} s^{-1} for the low-yielding cultivar (ASP-03) were observed in fully expanded cladophyll tissue measured in mid-summer (February) when both photon flux density (PFD) and temperature were at a maximum. A significant decline in A was measured in April, when plants experienced colder night temperatures and shorter day lengths. This was accompanied by a significant decrease in both stomatal conductance (g_s) and rubisco activity. These results indicate a tight coupling between g_s and biochemical capacity for CO_2 assimilation. A close correlation between A and g_s (r = 0.84) was observed. Although the correlation between A and total rubisco activity was rather weak (r = 0.26), both *in vivo* and fully activated rubisco activities in ASP-69 were significantly greater than in ASP-03, indicating the important role of this enzyme to cultivar differences in photosynthetic capacity. Timing of cladophyll initiation and duration did not appear to be significant factors contributing to cultivar difference in photosynthesis. Variation in photosynthetic capacity between the two cultivars was related to significant differences in cladophyll thickness and specific leaf weight (SLW). The results substantiate the conclusion that both metabolic and anatomical factors play significant roles in determining differences in photosynthetic capacity between the two asparagus cultivars studied.

The findings outlined in this chapter give some indication of photosynthetic capacity in relation to cultivar differences in spear yield. Of further interest is the process of photoassimilate allocation, as this is likely to provide insight into the regulation of carbohydrate partitioning. This is the focus of the following chapter.

CHAPTER 3

CARBON PARTITIONING AND SUCROSE METABOLISM IN CLADOPHYLL AND ROOT TISSUES

Chapter 3

Carbon partitioning and sucrose metabolism in cladophyll and root tissues

3.1 Introduction

Crop yield under field conditions is regulated by both genetic and environmental components which influence photoassimilate production and its partitioning (Gifford and Evans 1981; Daie 1985; David 1995). Studies in crop physiology to date have shown that the relationship between photosynthesis and crop yield is usually poor, whereas partitioning of assimilated carbon among the various growth and storage sinks during plant development is critical to the patterns of plant growth (Daie 1985; Barber 1994). Accordingly, there has been considerable interest in the elucidation of the regulatory mechanisms that are responsible for observed genetic differences in dry weight partitioning (Stitt *et al.* 1987; Huber *et al.* 1992; Sung *et al.* 1994). Some studies (Farrar and Williams 1991; Geiger *et al.* 1996; Pollock and Farrar 1996) have demonstrated that sucrose, the major carbohydrate transported in higher plants, not only supplies carbon for various processes in sink tissues, but is also involved in controlling growth. Since sucrose can provide carbon for cellular metabolism only after it has been converted to hexose phosphates (Sung *et al.* 1988), the timing and location of both sucrose synthetic and degradation enzyme activities can be important determinants of carbon partitioning through their effects on the balance between

photosynthetic and non-photosynthetic organs. Indeed, activity of sucrose phosphate synthase (SPS), the critical enzyme in sucrose synthesis, is often closely correlated with the rate of sucrose export by source tissue (Huber *et al.* 1992), whereas the activities of acid invertase (AI) and sucrose synthase (SS) are often related to the rate of sucrose import by sink tissue (Morris and Arthur 1984, 1985; Sung *et al.* 1994).

In asparagus, storage root tissue is the biggest sink for current photoassimilates (Benson and Takatori 1980; Wilson *et al.* 1999). Asparagus plants allocate a significant fraction of their photosynthetic output to long-term storage roots and after winter dormancy stored carbohydrate is utilised in spear growth during the next spring (Benson and Takatori 1980; Shelton and Lacy 1980; Woolley *et al.* 1999). Studies in asparagus physiology have shown a close relationship between the amount of root storage carbohydrate and spear yield (Robb 1984; Wilson *et al.* 1999). As spear harvest proceeds, root storage carbohydrates decline and are restored following fern development (Shelton & Lacy, 1980). Benson and Takatori (1980) reported that high-yielding asparagus cultivars had a higher percentage of root dry weight than low-yielding cultivars. In another study, Pressman *et al.* (1993) observed a significant cultivar difference in the levels of root storage carbohydrate throughout the season. Recently, both Bai and Kelly (1999) and Faville *et al.* (1999b) have reported a positive correlation between light saturated photosynthesis (A_{max}) and spear yield among some asparagus cultivars. In the current study, a close relationship between A_{max} and spear yield has also been found in the two cultivars studied. Moreover, both stomatal conductance and rubisco activity were associated with high yield (Chapter 2). These results may also be indicative of genetic differences in assimilate partitioning as carbon assimilation and partitioning are commonly closely coupled (Wardlaw 1990; Huber and Huber 1992). This relationship may be more obvious in asparagus as storage roots are a large carbohydrate sink which account for the majority of exported assimilates from source cladophylls (Faville *et al.* 1999a; Wilson *et al.* 1999; Woolley *et al.* 1999). In this context, seasonal variation in root storage carbohydrate and its metabolism may reflect a persistent relationship between

photosynthetic carbon gain and the activity of carbon sinks (Menke and Trlica 1981; Cyr *et al.* 1990; Schibata and Nishida 1993; Tissue and Wright 1995; Wyka 1999).

Despite the close link between carbohydrate storage and spear yield in asparagus, the relative roles of carbon metabolising enzymes in carbon partitioning and their relationship with spear yield have not been investigated. In this chapter, the hypothesis that cultivar difference in spear yield is associated with assimilate partitioning and thus with fluctuations in storage root carbohydrate content was tested. One objective of this chapter was to construct a profile of annual assimilate partitioning and sucrose metabolism to investigate the physiological parameters underlying cultivar differences in spear yield. The second was to investigate the roles of source-sink relationships in the regulation of carbon partitioning. No reports of the simultaneous monitoring of these related parameters in field grown asparagus were found.

3.2 Materials and methods

3.2.1 Plant growth and tissue harvesting

To examine carbohydrate storage and mobilisation in storage roots, source-sink relationships were modified by shading half of each plot. The shaded plants were covered with black shade cloth (70% shade) stretched across the shade area at a height of 2 m. Cladophyll tissue in the upper canopy was used for all the measurements. For the determination of seasonal patterns, measurements were made in the middle of the measuring days between 1100 and 1300 h because there was no midday depression of photosynthesis observed (Chapter 2).

At the end of March (mature cladophyll stage), measurements of plant height and shoot diameter were made. Two plants of each cultivar were harvested and washed to remove soil and separated into shoot, young storage roots and old storage roots. Tissue was dried for 24 hours at 90°C followed by 72 h at 70°C, and dry weight (DW) recorded. The samples (cladophylls and immature growing tips) destined for enzyme determinations were quickly weighed and immediately frozen in liquid nitrogen and then stored at -80°C prior to assay, whereas the samples for carbohydrate content determination were freeze-dried. Three replicates for each cultivar were collected. Storage roots were harvested at midday. They were washed with tap water, rinsed with distilled water and wiped. The roots were then cut into small pieces (0.5 cm long) and randomly sub-sampled up to a fresh weight of 1 g. The fresh samples were stored at -80°C prior to carbohydrate extraction.

3.2.2 A_{max} and carbohydrate determination

Maximum photosynthetic rate (A_{max}) under ambient PFD conditions was calculated from diurnal patterns of photosynthesis measured on individual days each month as described in chapter 2. Soluble sugar and starch content of cladophylls and storage roots were determined according to Tissue and Wright (1995). Frozen tissue of 0.5 g fresh weight (FW) was extracted by grinding in 2 mL of methanol:chloroform:distilled water (12:5:3) for 30 min in a shaking bath at a 25°C to separate soluble sugar from starch. This homogenate was centrifuged at 11,000 × *g* for 5 min and the supernatant was transferred to a 25-mL test tube. The pellet was resuspended twice in 2 mL of extraction mixture and centrifuged. The three supernatants were combined, 2 mL H_2O added, mixed thoroughly and placed overnight in a refrigerator. Supernatant of 50 µL was transferred to a fresh test tube, 200 µL H_2O, 250 µL 5% phenol and 1.25 mL H_2SO_4 added and mixed thoroughly. After cooling, the soluble sugar was determined by absorbance at A_{490} against distilled water. The pellet for starch determination was left to dry in a fume-hood overnight, and then 5 mL of 35% perchloric acid was added to digest polysaccharides into

sugars. After shaking for 60 min at room temperature, the mixture was filtered and 50 μL was transferred into a fresh tube. This sample was assayed for soluble sugar as above. Hexose sugar content was determined by quantifying glucose formation assayed in a 1 mL mixture containing 100 m*M* BES plus 0.4 m*M* NADP, 1 m*M* ATP, 0.5 unit hexokinase, 1 unit phosphoglucoisomerase, 2 units glucose-6-P dehydrogenase, 5 m*M* $MgCl_2$, 0.5 m*M* EDTA, 0.02% BSA (w/v) and 20 μL of sample extract. Sucrose content was determined after hydrolysing the sucrose present in the extract with AI (Sigma I 4504) and then estimating the glucose released (William *et al.* 1988).

The percentage of soluble sugar in the stem cell sap (obtained by freezing-thawing and expressing) was analyzed using a Digital Refractometer (Palette 100, Atago Co., Ltd. Tokyo 173, Japan).

3.2.3 Enzyme extraction and assay

Frozen cladophyll or storage root tissue (1g fresh weight, FW) was extracted by grinding in a pre-cooled mortar using 5 mL of extraction buffer (pH 7.5) containing 50 m*M* HEPES, 50 m*M* $MgCl_2$, 10 m*M* EDTA, 10 m*M* EGTA, 2.5 m*M* DTT and 0.1% Triton X-100. The homogenates were centrifuged 11,000 × g for 5 min at 4°C in 10 mL centrifuge tubes. The supernatant was immediately desalted in Sephadex G-25 (Sigma Chemical Co.) columns equilibrated with extraction buffer minus the Triton X-100. Activities of SPS, AI and SS were assayed immediately after desalting.

SPS activity was assayed under limited (V_{lim}) and saturated (V_{max}) substrate conditions via F-6-P dependent formation of sucrose (plus sucrose-P) from UDPG (Huber and Huber 1991). Under V_{lim} conditions, 20 μL tissue extract was incubated 10 min at 25°C with 10 m*M* UDPG, 2.5 m*M* F-6-P, 10 m*M* G-6-P (an activator), 10 m*M* Pi (an

inhibitor), 50 m*M* HEPES-KOH (pH 7.5), 15 m*M* $MgCl_2$ and 1 m*M* EDTA in a total volume of 70 µL. Under V_{max} conditions, the substrates F-6-P and G-6-P were increased to 10 m*M* and 40 m*M* respectively and the inorganic phosphate was removed from the reaction mixture, but the total volume and reaction time were same as the limiting assays. All reactions were terminated by the addition of 70 µL 30% KOH and unreacted F-6-P was destroyed by placing the tubes in boiling water for 10 min. After cooling, 2 mL of 0.14% anthrone in 13.8 M H_2SO_4 was added and the tubes were incubated at 40°C for 20 min prior to measuring absorbance at A_{620}. SPS activity of each sample was calculated from the rate of change in sucrose concentration. The ratio of two activities (V_{lim}/V_{max}) was considered as the activation state and was expressed as a percentage.

AI activity was assayed according to Kalt-Torres and Huber (1987). A reaction mixture (240 µL total volume) contained 20 µL of desalted enzyme extract, 20 µL of sucrose (50m*M*) and 200 µL of citrate-phosphate buffer (100 m*M* and pH 5.0). Reactions were initiated by addition of the enzyme extract and incubated at 25°C for 30 min, and stopped by boiling the tubes at 100°C for 10 min. The activity of AI was measured by quantifying the amount of glucose (Albert *et al.* 1988) released from hydrolysis by AI in comparison with the blank in which the invertase was killed by heating at 100°C for 5 min prior to the assay. SS activity was measured in the synthetic direction by quantification of sucrose formation using the anthrone method (Huber *et al.* 1996). Assays (70 µL) contained 7.0 µmol fructose, 10 µmol UDP-glucose dissolved in 50 m*M* Hepes-NaOH (pH 7.5), 15 µmol $MgCl_2$ plus 20 µL desalted extract. The reaction was terminated by the addition of 70 µL of 30% (w/v) KOH and immersion of tubes in a boiling water bath for 10 min. After the samples were cooled, 2 mL of 0.15% (w/v) anthrone in H_2SO_4 was added, the tubes were incubated at 40°C for 20 min, and the A_{620} was recorded. Sucrose formation was calculated by comparison to a sucrose standard curve after subtraction of a blank in which SS was killed by heating at 100°C for 5 min prior to the assay. Both AI and SS activities were linear with time and proportional to the amount of extract added.

3.2.4 ^{14}C pulse-chase labelling of intact ferns

Intact field-grown ferns were used to trace ^{14}C partitioning into storage roots. The concept of the physiological unit introduced by Hughes (1992) was adopted. According to this concept, the physiological unit in carbon assimilation and partitioning is confined to the individual rhizome and associated ferns and roots. The validity of this concept has been supported by the findings of Faville *et al.* (1999a) and Woolley *et al.* (1999). Two replicate ferns from each cultivar were used in this experiment. The fern was enclosed in a plastic bag and photosynthetically labelled with 100 μCi ^{14}C ($Na^{14}CO_3$). The method of feeding ^{14}C to plants was according to that of Flora and Madore (1996). ^{14}C was released by adding an adequate amount of lactic acid with a syringe into a beaker containing $Na^{14}CO_3$ solution which was suspended in the bag. After 30 min of exposure to^{14}C the bag was removed. After a two-day chase period, plants were harvested and dried. A dry sample of 100 mg was extracted as for carbohydrate analysis and radioactivity was measured in a liquid scintillation counter (Packard 2500 TR; Canberra-Packard, Dreieich, Germany) after adding 2 ml of scintillation cocktail (Aquasafe 300 plus; Zinsser Analytic, Frankfurt, Germany) to the 50 μL extract solution.

3.2.5 Statistical analysis

All growth data presented are the means ± SE of 12 replicates except biomass data that are the means ± SE of 2 replicates. The biochemical data are means ± SE of 3 replicates. One way analysis of variance (ANOVA) was used to test for difference between the two cultivars. Differences were considered significant if $P < 0.05$.

3.3 Results

3.3.1 Plant biomass

Plant height and shoot diameter data are given in Table 3.1. ASP-69 displayed greater shoot height and diameter in comparison to ASP-03. Shoot shading in the previous season significantly reduced plant height and shoot diameter in both cultivars in the following season. Statistical analysis revealed a significantly higher ($P < 0.05$) percentage of young storage roots to total biomass in ASP-69 (3.8 ± 0.17 in %) than in ASP-03 (2.4 ± 0.19). The percentage of shoot to total biomass in ASP-69 (15.1 ± 0.14) was lower than that in ASP-03 (19.0 ± 0.58), although this was not significantly different. The root/shoot ratio was, therefore, greater in ASP-69 (5.6 ± 0.06) than in ASP-03 (4.3 ± 0.14).

3.3.2 Seasonal changes in A_{max} and carbohydrate content

The two cultivars displayed similar seasonal patterns in A_{max}, which increased initially, reached a maximum in fully expanded cladophyll tissue in February and then declined during the rest of the season (Fig. 3.1). ASP-69 displayed significantly greater A_{max} in fully expanded cladophyll tissue, but not in expanding (January), mature (March) or senescent cladophyll tissues (April and May).

	Growth	
	Plant height (cm)	Shoot diameter (mm)
ASP-69		
Control	175 ± 4.6	12.2 ± 0.58
Shaded	139 ± 6.3	8.9 ± 0.58
ANOVA	***	***
ASP-03		
Control	120 ± 4.6	8.9 ± 0.56
Shaded	71 ± 5.4	6.2 ± 0.62
ANOVA	***	***

Table 3.1. Effects of shading in the previous season on plant height and shoot diameter in the following season. Results are means of 12 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated: ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

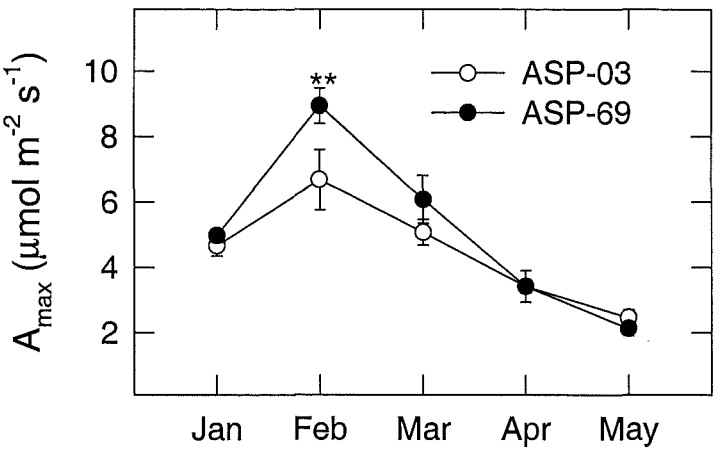


Fig. 3.1. Seasonal changes in maximum photosynthetic rate (A_{max}) in ASP-69 (closed symbol) and ASP-03 (open symbol). Results are means of 6 replicates ± SE. Significant differences between ASP-69 and ASP-03 are indicated as ** $P < 0.01$.

Changes in TNC in cladophyll tissue at different developmental stages are shown in Fig. 3.2a. As cladophyll expansion progressed, TNC content increased, reaching a maximum of 251 ± 5 mg g^{-1} DW for ASP-69 and 213 ± 12 mg g^{-1} DW for ASP-03 in fully expanded tissue in February and then remained constant until April. In late senescent tissue in May, TNC in both cultivars declined. The major sugar detected in the cladophyll tissue was sucrose, which constituted 50-60% of the carbohydrate pool in fully expanded and mature cladophyll tissue (Fig. 3.2b), whereas starch and hexose accounted for approximate 20% and 10%, respectively (Fig. 2c,d). In expanding

cladophyll tissue, sucrose and hexose contents were initially about equal, after which there was a continued rise in sucrose content while hexose content declined with time (Fig. 3.2b,d). After full cladophyll expansion, hexose content declined further to a minimum in senescent tissue (Fig. 3.2d).

In both cultivars, TNC content in storage roots remained constant during the winter while the plant was dormant (Fig. 3.3a). During the spear production period, a depletion of storage root TNC took place, using approximately 20-25% of storage reserve. Shoot establishment following spear harvest was accompanied by a more severe depletion in TNC, leading to a mean seasonal minimum in TNC of 184 ± 8 mg g^{-1} DW for ASP-69 and 194 ± 10 mg g^{-1} DW for ASP-03 (Fig. 3.3a). Carbohydrate replenishment started in the early cladophyll expansion stage. Sucrose content in storage roots was lower than in cladophyll tissue and did not differ significantly between the two cultivars (Fig. 3.3b). During winter dormancy, sucrose content tended to increase and then declined during spear production and fern establishment, after which there was a continuous rise with further fern development. By contrast, hexose concentration decreased through the winter dormant period and spear harvest phase and then increased sharply as cladophyll development progressed (Fig. 3.3c). During this period, hexose content in ASP-69 was significantly greater than in ASP-03. After cladophyll maturation, hexose content declined and did not differ between the two cultivars. In the stems measured at the mature cladophyll stage (March), ASP-69 possessed a significantly greater soluble sugar content than did ASP-03 (Fig. 3.4). Cell sap expressed from stem showed a significantly higher percentage of soluble sugar in ASP-69 (8.68 ± 0.89 in %) than in ASP-03 (5.84 ± 1.01) (Fig. 3.4).

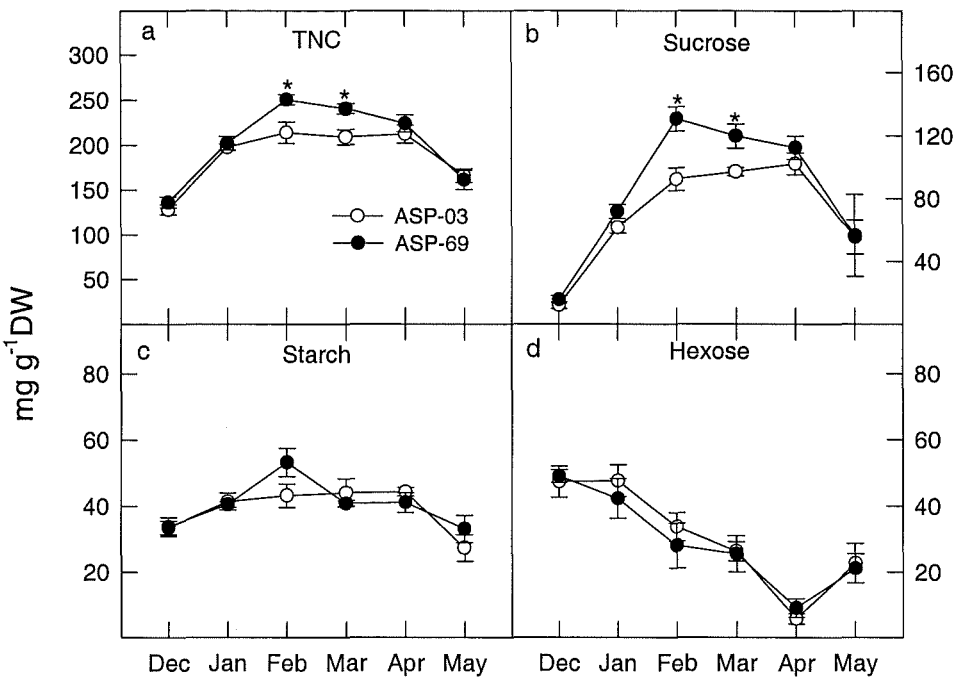


Fig. 3.2. Seasonal changes in (a) total non-structural carbohydrate (TNC), (b) sucrose, (c) starch and (d) hexose concentrations in cladophyll tissues of two field-grown asparagus cultivars (ASP-69, closed symbol; ASP-03, open symbol). Dec. and Jan. = expanding cladophyll tissue; Feb. = fully expanded cladophyll tissue; Mar. = mature cladophyll tissue; Apr. and May = senescent cladophyll tissue. Results are means of 3 replicates ± SE. Significant differences between ASP-69 and ASP-03 are indicated as * $P < 0.05$.

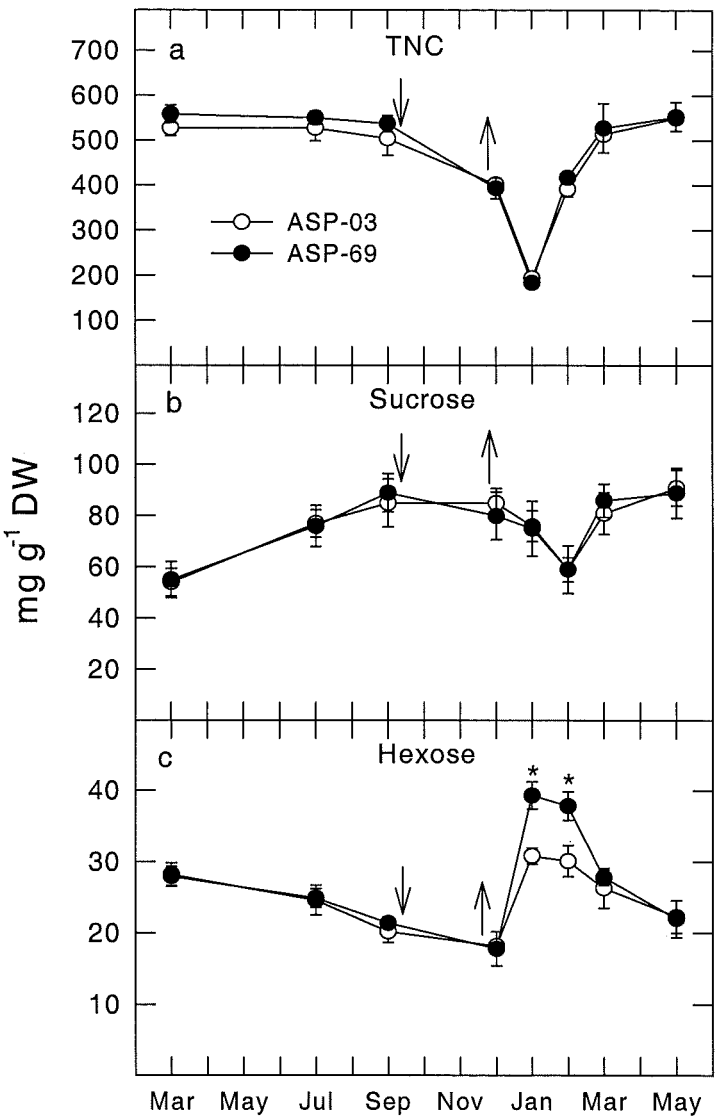


Fig. 3.3. Seasonal changes in (a) TNC, (b) sucrose and (c) hexose concentrations in storage root tissue of two field-grown asparagus cultivars (ASP-69, closed symbol; ASP-03, open symbol). Arrows indicate the spear harvest period. Results are means of 3 replicates ± SE. Significant differences between ASP-69 and ASP-03 are indicated as * $P < 0.05$.

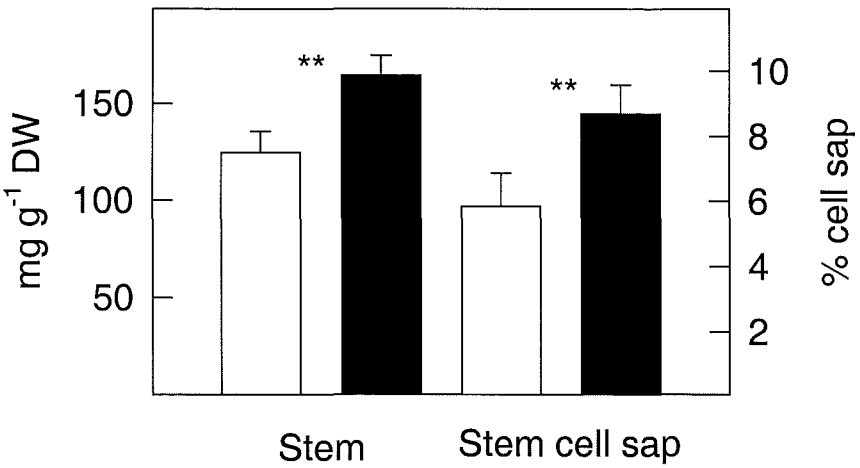


Fig. 3.4. Soluble sugar content in stem tissue and percentage of soluble sugar in stem cell sap of two field-grown asparagus cultivars (ASP-69, closed bar; ASP-03, open bar) measured at the mature cladophyll stage. Results are means of 6 replicates ± SE. Significant differences between ASP-69 and ASP-03 are indicated as ** $P < 0.01$.

3.3.3 Effect of shading on root carbohydrate storage and usage

Table 3.2 summarises the effects of cladophyll shading on carbohydrate accumulation and mobilisation in cladophyll and storage root tissues. Cladophyll shading significantly reduced TNC in mature cladophyll tissue by approximately 25% in ASP-69 and 28% in ASP-03 (Table 3.2). A similar trend was also found in the storage root

reserve by the end of the fern growth season. TNC was reduced from 527 ± 55 to 378 ± 18 mg g^{-1} DW in ASP-69 and 513 ± 13 to 354 ± 14 mg g^{-1} DW in ASP-03 (Table 3.2). The decline in TNC under the shading treatment was accompanied by a decrease in cladophyll sucrose content of 20%. Similarly, average sucrose content declined by 30% in the storage roots of both cultivars. During cladophyll development in the second season after shading, TNC in storage roots depleted to the same levels in shaded and unshaded controls, indicating that a fixed proportion of reserve is not available for utilization.

3.3.4 Seasonal changes in activity of sucrose metabolising enzymes

In cladophyll tissue, activities of AI and SS (sucrose degradation enzymes) exhibited similar seasonal patterns in the two cultivars (Fig. 3.5). Both AI and SS were high initially and then decreased with cladophyll maturation. There was no significant difference between the two cultivars although ASP-69 displayed slightly higher AI and SS activities in early season expanding cladophyll tissue. However, in immature growing tips measured in January, ASP-69 displayed significantly greater AI (117 ± 7.6 μmol h^{-1} g^{-1} FW) and SS (75.7 ± 3.3 μmol h^{-1} g^{-1} FW) activities than in ASP-03 (89 ± 6.9 and 57.7 ± 5.9 μmol h^{-1} g^{-1} FW for AI and SS respectively). In storage root tissue, activity of AI remained constant and did not differ significantly between the two cultivars throughout the season (Fig. 3.6a). In contrast, activity of SS remained unchanged during winter dormancy and then increased significantly during cladophyll development until cladophyll full expansion (Fig. 3.6b). In April and May SS activity declined with the onset of cladophyll senescence. ASP-69 displayed a significantly greater SS activity than ASP-03 during the cladophyll expansion phase. In both cultivars, SPS activity, both substrate limited (V_{lim}) and substrate saturated (V_{max}), exhibited a pattern opposite to that of SS and AI during cladophyll expansion. After reaching a maximum in the fully expanded cladophyll tissue, both V_{lim} and V_{max} remained constant in the mature cladophyll tissue and than declined following the onset of senescence (Fig. 3.7a,b). V_{lim} activity, which reflects *in vivo* activity, was

significantly greater in ASP-69 than in ASP-03 in the fully expanded and mature cladophyll tissues (Fig. 3.7a). A close correlation between A_{max} and SPS activity was found in both cultivars (r = 0.86).

3.3.5 Partitioning of ^{14}C-labelled photoassimilates to the storage roots

To determine whether carbohydrate partitioning was associated with cultivar difference in spear yield, a pulse-chase experiment was performed. Effort was made to obtain ^{14}C partitioning data based on individual rhizomes, but it was impossible to completely separate storage roots between rhizomes. Nevertheless, analysis of ^{14}C recovered in the storage root and stems following labelling suggested a relatively higher translocation rate in ASP-69 than in ASP-03. ^{14}C recovered in storage root tissues after 48 h averaged 2.18 ± 0.23 CPM 10^{-4} g FW for ASP-69 and 1.47 ± 0.17 CPM 10^{-4} g FW for ASP-03, respectively. These results were consistent with the results derived from stem cell sap analysis.

	Current shading season				Following season	
	Cladophyll		Storage root		Storage root	
	TNC (mg g^{-1} DW)	Sucrose (mg g^{-1} DW)	TNC (mg g^{-1} DW)	Sucrose (mg g^{-1} DW)	TNC (mg g^{-1} DW)	Sucrose (mg g^{-1} DW)
ASP-69						
Control	240 ± 6.1	120 ± 7.8	527 ± 55	86 ± 6.5	184 ± 8.8	80 ± 9.3
Shaded	180 ± 5.3	97 ± 4.3	378 ± 18	56 ± 5.3	192 ± 6.9	72 ± 5.4
ANOVA	*	*	***	*	ns	ns
ASP-03						
Control	209 ± 8.4	97 ± 2.6	513 ± 13	81 ± 8.1	194 ± 9.6	85 ± 5.7
Shaded	151 ± 13.4	78 ± 8.1	354 ± 14	58 ± 5.2	204 ± 11.6	80 ± 8.6
ANOVA	*	*	***	*	ns	ns

Table 3.2. Effects of shading on carbohydrate accumulation in mature cladophyll tissue and storage roots of two field-grown asparagus cultivars. Results are means of 3 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between shaded and unshaded controls are indicated: ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

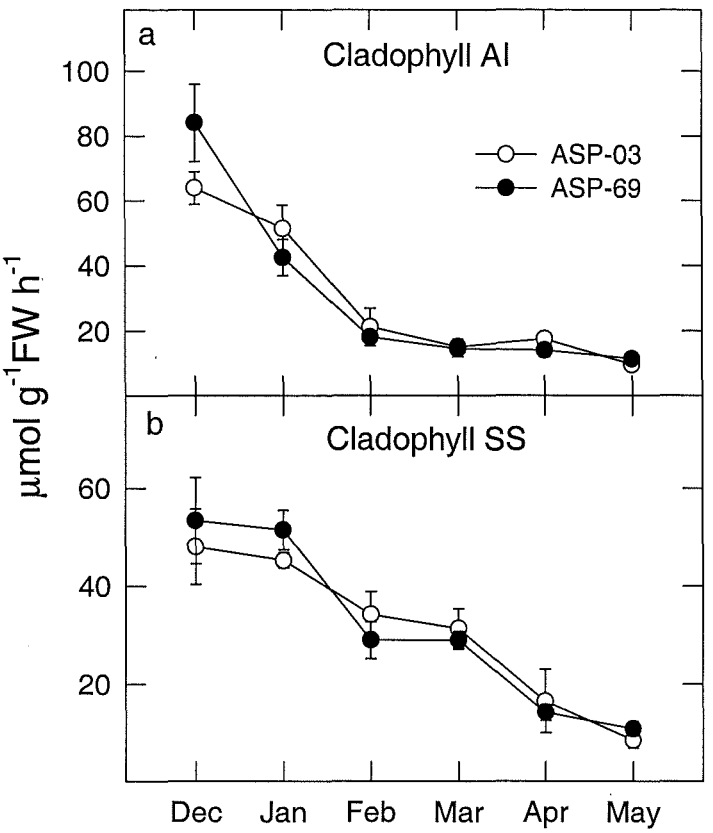


Fig. 3.5. Seasonal changes in (a) sucrose synthase (SS) and (b) acid invertase (AI) activities in developing cladophyll tissues of two field-grown asparagus cultivars (ASP-69, closed symbol; ASP-03, open symbol). Results are means of 3 replicates ± SE.

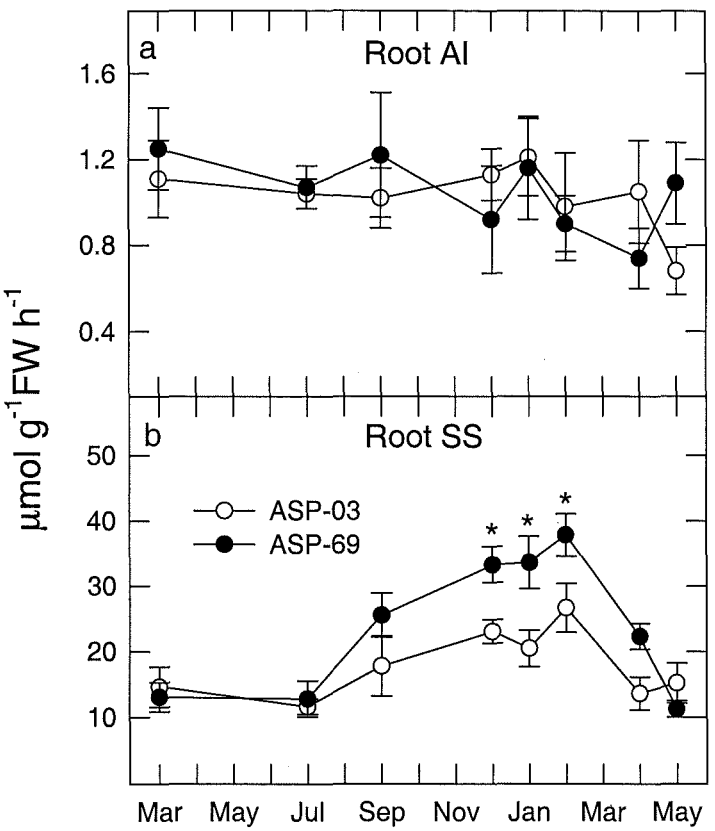


Fig. 3.6. Seasonal changes in (a) sucrose synthase (SS) and (b) acid invertase (AI) activities in storage root tissues of two field-grown asparagus cultivars (ASP-69, closed symbol; ASP-03, open symbol). Results are means of 3 replicates ± SE. Significant differences between ASP-69 and ASP-03 are indicated as * $P < 0.05$.

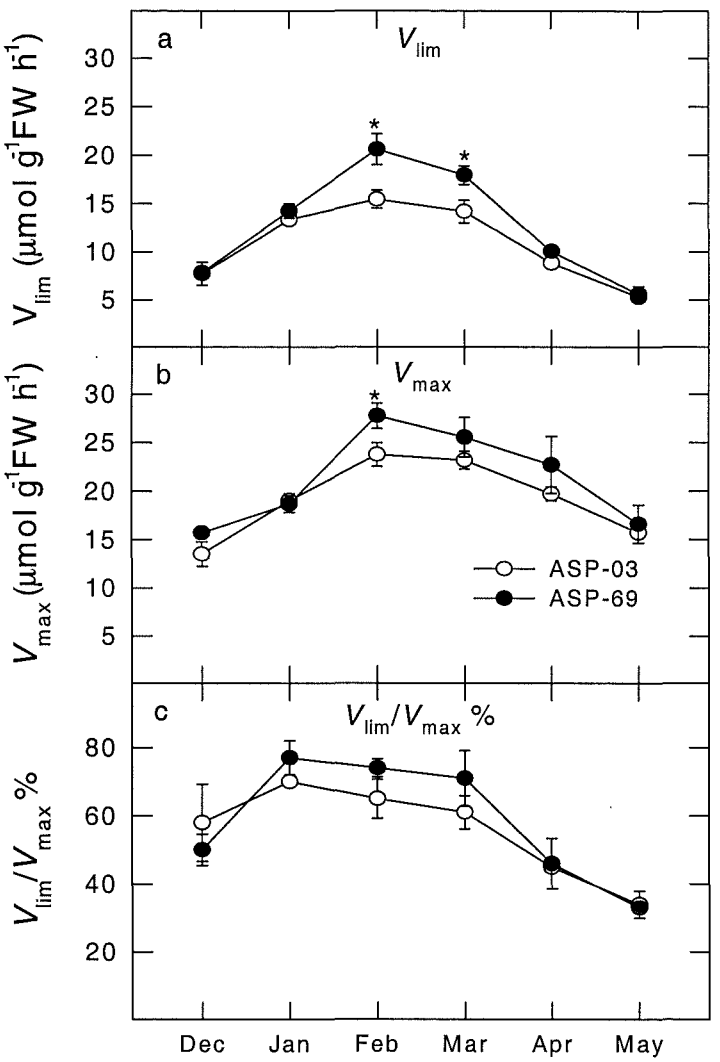


Fig. 3.7. Seasonal changes in sucrose phosphate synthase (SPS) activities in developing cladophyll tissues of two field-grown asparagus cultivars (ASP-69, closed symbol; ASP-03, open symbol). V_{lim} = substrate limited; V_{max} = substrate saturated. Results are means of 3 replicates ± SE. Significant differences between ASP-69 and ASP-03 are indicated as * $P < 0.05$.

3.4 Discussion

Carbon partitioning is generally considered to be a process influenced by both carbon supply in the source tissue and carbon demand among various sinks competing for the limited carbon supply (Foyer 1987; Farrar 1996). In sucrose transporting plants, sucrose content and its metabolism in source and sink tissues can play a significant role in the regulation of carbon partitioning (Wardlaw 1990; Sung *et al.* 1994). The results obtained in the present study indicate a close relationship between A_{max} and SPS activity in asparagus cladophyll tissue. In addition, high-yielding cultivar ASP-69 displayed greater A_{max}, SPS activity and sucrose concentrations than in the low-yielding cultivar ASP-03 in fully expanded and mature cladophyll tissue. Conversely, no cultivar difference in carbohydrate concentration in storage roots were measured, although ASP-69 possesses a greater root/shoot ratio, indicating that it is the total storage carbohydrate pool rather than carbohydrate concentration that is a major determinant of asparagus yield.

3.4.1 Effect of cladophyll age on sucrose metabolism

In most higher plants, growing leaves are initially sinks depending on import of sucrose for carbon into metabolism, and mature leaves are net exporters of sucrose (Hampp *et al.* 1994). Transition from sink to source during leaf development is mirrored by changes in activity of enzymes involved in sucrose cleaving (AI and SS) and synthesis (SPS) (Huber *et al.* 1986). However, the timing and relative activity of sucrose enzymes during this transition are species dependent (Huber and Israel 1982; Claussen *et al.* 1985; Hampp *et al.* 1994). In the current study, the rapidly expanding cladophyll tissue was characterised by a rapid increase in SPS activity, accompanied by an increase in sucrose content, indicating a rapid sink to source transition. Storage root carbohydrate content was depleted further during this period. These results are

consistent with those of Woolley *et al.* (1999) who, using ^{14}C to trace the current assimilates, reported that 70% of assimilated ^{14}C was allocated to growing shoots in the early stage of cladophyll development. Thus, both storage carbohydrate reserve and current photoassimilates may contribute to the late stage of shoot development.

The period from fully expanded to mature cladophyll tissue was the phase in which greater photosynthesis and SPS activity were found and approximately 95% of storage carbohydrate was replenished. Moreover, the greatest cultivar differences in carbohydrate metabolism also occurred in this period. With the onset of cladophyll senescence, SPS activity decreased rapidly in both cultivars, presumably in response to cold nights and short days (Robb 1984). However, carbohydrate accumulation remained relatively constant until the advanced senescence stage, indicating a lower sink demand. These results suggest that the timing of cladophyll initiation and duration may not be significant factors contributing to differences in spear yield between the two cultivars studied.

3.4.2 Carbohydrate storage and usage in root tissue

The importance of storage root carbohydrates on shoot growth is indicated by the reduced shoot diameter and height in both cultivars following cladophyll shading during the previous season. Shading caused a significant decrease in carbohydrate content in storage roots. However, the amount of carbohydrate present in storage roots at the end of fern establishment in the second season did not differ between shaded and unshaded controls, indicating that the remaining carbohydrates are not available to the plant for withdrawal. It has been suggested that bud number in asparagus is genetically controlled, whereas subsequent growth into a mature fern is dependent primarily on the availability of storage root carbohydrate (Robb 1984; Drost and Wilcox Lee 1997a). In this regard, the amount of available carbohydrate following the shading treatment may have been insufficient to support shoot growth or it may have

induced carbohydrate starvation of buds during development. Since control plants in ASP-69 had a significantly greater shoot diameter than those of ASP-03 while the carbohydrate concentration in storage roots was not significantly different, it is unlikely that content of storage carbohydrate was responsible for the observed difference in shoot size. In some studies, it has been shown that carbohydrates stored in specific parts of the root system are used for specific stages of growth (Faville *et al.* 1999a; Wilson *et al.* 1999; Woolley *et al.* 1999). For example, Faville *et al.* (1999a) reported that assimilated ^{13}C is concentrated most strongly in the bud and new bud roots, and is utilised during bud emergence. Our results support this hypothesis. Firstly, greater assimilation rate in ASP-69 was associated with a greater shoot diameter in comparison to ASP-03. Secondly, shading reduced shoot diameters in both cultivars. Thirdly, the phase in which assimilation rate reached a maximum in the fully expanded cladophyll tissue accompanied that of active new root production. Taken together, these results suggest that bud size is limited primarily by the newly fixed assimilates allocated to the bud roots, whereas overall shoot growth is related to the total available carbohydrate reserve.

3.4.3 Source-sink impacts on carbon partitioning

While there is considerable evidence to support the concept that carbon partitioning is regulated by source-sink relations (Gifford and Evans 1981; Daie 1985; Patrick 1988; Daie 1996), the mechanisms underlying assimilate partitioning are still only poorly understood (Wardlaw 1990; Marcelis 1996). Some studies have indicated strongly that carbohydrate partitioning among sinks is primarily regulated by the sinks themselves (Daie 1985; Balibrea *et al.* 2000). However, other studies have shown that the source can exert great influence on carbon partitioning (Fader and Koller 1983; Grodzinski *et al.* 1998; Noormets *et al.* 2001). In asparagus, the storage root is the major sink for current photoassimilates except in the stage of fern establishment (Wilson *et al.* 1999; Woolley *et al.* 1999). Previous studies (Bai and Kelly 1999; Faville *et al.* 1999b) and also this study (Chapter 2) have shown a close relationship

between photosynthesis and spear yield among some asparagus cultivars, suggesting a feed-forward relation between carbon assimilation and partitioning into storage roots. In addition, a close relationship between A_{max} and SPS activity was observed in cladophyll tissues in the two cultivars studied. These results suggest a significant role of source tissue on carbon partitioning.

The biomass of storage roots in asparagus is relatively stable for mature plants, and is balanced by the senescence of old storage roots and growth of new storage roots at the time of fern development (Culpepper and Moon 1939a; Robb 1984). In the current study, greater SPS activity in ASP-69 was associated with greater carbon partitioning to storage roots (as indicated by a greater root/shoot ratio). These results suggest that sink strength rather than sink capacity is responsible for attracting incoming assimilates and preventing any feedback effect on carbon partitioning. The metabolic changes in SS activity in storage roots were consistent with changes in hexose content during fern growth season. In this regard, the rise in SS activity may be related to the unloading of sucrose translocated from the shoots or regrowth of storage roots, since it has been reported that SS activity is usually associated with sink demand (Sung *et al.* 1989). Thus, both SS activity and the size of the carbohydrate pool exert a significant influence on carbon partitioning. It is concluded that carbon partitioning from source cladophyll to storage sink is a property controlled by both source and sink strength.

3.4.4 Cultivar variation in carbon partitioning and sucrose metabolism

Although fern vigour of asparagus has often been found to correlate with spear yield (Robb 1984), it is uncertain how these two parameters are connected as greater assimilate production does not necessarily lead to greater yield (Patrick 1988). ASP-69 not only has a greater total biomass but also a greater root/shoot ratio than ASP-03, whereas carbohydrate concentration in storage root tissue was not significantly

different between the two cultivars. An allocation of carbon in favour of dry matter increase rather than an increase in carbon concentration in the storage roots may partly contribute to the greater spear yield in ASP-69. Apart from significant differences in the total carbohydrate storage pool between the two cultivars, significant differences were also found in sucrose metabolism. ASP-69 exhibited greater AI and SS activity than in ASP-03 in the immature growing tips, consistent with the finding that ASP-69 possesses a greater shoot elongation rate than in ASP-03 (Chapter 5). In some species, it has been shown that the hydrolysis of sucrose in sink tissue may determine the ability to import photoassimilates (Balibrea *et al.* 2000) and that the activity of sucrose cleaving enzymes could be used as biochemical indicator of sink strength (Sung *et al.* 1994). Current results suggest that greater canopy size observed in ASP-69 than in ASP-03 is related to SS and AI activities in the growing tips. In the fully expanded and mature cladophyll tissues, ASP-69 displayed significantly greater A_{max} and SPS activity than in ASP-03, consistent with a greater sucrose concentration in the cladophyll tissue. Together with the relatively lower sucrose concentrations in the storage roots, a greater carbon translocation rate in ASP-69 is expected. Indeed, both stem cell sap and ^{14}C analysis were in agreement with this result. Furthermore, ASP-69 displayed a greater SS activity in the storage root than in ASP-03 during the fern development phase. These results suggest strongly that both source (cladophyll) and sink (storage roots) play significant roles in regulating carbon partitioning in the two cultivars studied and that the high-yielding cultivar ASP-69 displayed a more efficient carbon partitioning strategy than the low-yielding cultivar ASP-03.

3.5 Summary

The aim of this chapter was to investigate the roles of carbon partitioning and sucrose metabolism in regulating cultivar differences in yield in asparagus (*Asparagus officinalis* L.). In the two cultivars studied, maximum photosynthetic rate (A_{max}) was positively correlated with sucrose phosphate synthase (SPS) activity (r = 0.86). The

high-yielding cultivar ASP-69 exhibited greater SPS activity and sucrose content than the low-yielding cultivar ASP-03 in fully expanded and mature cladophyll tissue. ASP-69 also displayed a higher percentage of soluble sugar in stem cell sap than did ASP-03. These results suggest that carbon translocation rate in ASP-69 is higher than in ASP-03. Sucrose synthase (SS) activity in storage roots in ASP-69 was significantly greater than in ASP-03 during fern growth season. Total non-structural carbohydrate (TNC) in storage roots did not differ in the two cultivars. Biomass analysis revealed that ASP-69 had a greater root/shoot ratio than in ASP-03, suggesting that the total carbohydrate storage pool rather than carbohydrate concentration is an important determinant of asparagus yield. The overall results substantiate the conclusion that carbohydrate partitioning in the two asparagus cultivars studied is a property of the entire plant and is influenced by both source and sink properties. This is highlighted by greater A_{max}, SPS activity and sucrose concentrations in cladophyll tissue in ASP-69 and greater SS activity and total carbohydrate content in storage root tissue in ASP-69.

The results observed in chapter 2 and 3 are likely to have important physiological consequences for the coordination between carbon assimilation and its partitioning into storage roots, and it is to such considerations that the following chapter will be directed.

CHAPTER 4

CARBON ASSIMILATION, PARTITIONING AND EXPORT IN MATURE CLADOPHYLL TISSUE

Chapter 4

Carbon assimilation, partitioning and export in mature cladophyll tissue

4.1 Introduction

While the relationship between rate of photosynthesis and agricultural yield is uncertain, it is clear that, for a given plant, total plant productivity will depend on appropriate export of carbon from source leaves (Komor 2000). In some plants, a linear relationship between carbon assimilation, partitioning and export in source leaves is observed. For example, Fader and Koller (1983) reported a positive correlation between photosynthetic rate, sucrose concentration and export rate in soybean leaves. More recently, Grodzinski et al. (1998) examined 21 C_4 *vs* C_3 species by ^{14}C labelling of leaves and found that carbon export rate was closely correlated with photosynthetic rate and sucrose concentration. However, conflicting results have also been reported in some species. For example, Webb (1969) reported that leaf sucrose concentrations have no correlation with rates of export in *Cucurbita melopepo*. It is hypothesized that in the latter case, it may not be the sucrose concentration but rather its delivery to the phloem that is important in determining carbon export rates (Mitchell *et al.* 1992). Thus, different species may adopt different strategies which influence the relationship between rate of carbon assimilation and its export.

Results presented in chapter 2 and 3 have confirmed findings of Faville *et al.* (1999a,b) and Bai and Kelly (1999) who reported that A_{max} was positively associated with spear yield and the majority of fixed carbon was allocated into storage roots during mature cladophyll stage. Similar results have also been reported by Benson and Takatori (1980) who observed a higher root:shoot ratio in a high-yielding cultivar than in the low-yielding cultivar. Clearly, a large proportion of total assimilate is required for both new storage root development and storage during the fern growth season in order to meet the requirement of spear development at the next season (Haynes 1987; Woolley *et al.* 1999). These results suggest that it may be source supply rather than sink demand that plays a major role in the regulation of carbon export out of cladophyll tissue in asparagus. However, despite the importance of photosynthate translocation to asparagus yield, very little information is available concerning assimilate export in relation to carbon assimilation and carbon metabolism in cladophyll tissue. The results obtained from chapter 2 have shown a significant difference in the rate of carbon assimilation between the two asparagus cultivars (ASP-69 and ASP-03) with contrasting yield. It would be expected that such differences in assimilation rate may lead to cultivar differences in assimilate partitioning and export. In this chapter, a comparison of diel patterns of carbon assimilation, partitioning and export from mature cladophyll tissue in two asparagus cultivars is made. The objective was to test the hypothesis that carbon export rate in mature cladophyll tissue is associated with carbon assimilation rate and sucrose availability. An attempt was made, firstly, to construct a profile of allocation of carbon between sucrose and starch to address the question: do both sucrose and starch have significant roles contributing to carbon export in a day/night cycle? Secondly, an attempt was made to resolve the question: are rates of export directly linked to the rates of carbon assimilation and sucrose concentration in the cladophyll tissue? The significance of these findings in explaining differences in asparagus yield is considered.

4.2 Materials and methods

4.2.1 CO_2 assimilation measurements

Natural diurnal courses of gas exchange were measured on three plants for each cultivar. Cladophyll photosynthetic rate (*A*) was determined using a portable gas analysis system (Li-Cor model 6400, Lincoln, NE, USA) equipped with a CO_2 control module as described in chapter 2.

4.2.2 Cladophyll dry matter and carbohydrate determination

Dry matter accumulation per unit cladophyll area during the course of the day was measured as changes in cladophyll dry weight. This technique assumes no significant changes in cladophyll area for mature tissue throughout the measurements. This was confirmed by measuring and monitoring cladophyll area through the day/night cycle using imaging software (MetaMorth version 4.0, Universal Imaging Corporation). Six replicates (40 cladophylls each) were removed from 2 ferns × 3 plants for each cultivar at three-hour intervals. To reduce variation between ferns and potential wounding artifacts caused by the sampling techniques, same age ferns were used to collect the samples. A pre-examination confirmed this technique and indicated that there was little difference in photosynthetic capacity and carbohydrate content in the mature cladophylls selected within a plant. At each sampling time, two end branches bearing cladophylls were cut from the base of the branch and 20 cladophylls counted. A total of 40 uniform cladophylls were used in one determination. Cladophylls were weighed and quickly frozen in liquid nitrogen in aluminium foil envelopes within two minutes of excision. The cladophylls were then freeze-dried for 48 h and re-weighed to obtain dry weight. Total non-structure carbohydrate (TNC), soluble sugar, starch were determined according to Tissue and Wright (1995), whereas sucrose and hexose contents were determined according to William *et al.* (1988) as described in chapter 2.

4.2.3 Estimation of assimilate export

Assimilate export rates were estimated by the method of Fader and Koller (1983). The rate of export T was determined using the relationship

$$T = P - A$$

Where P is the calculated rate of carbohydrate production due to CO_2 fixation and A is the rate of accumulation of dry matter. It was assumed that the dry matter changes in the cladophyll were attributed to changes in carbohydrate compounds. In order to express assimilation rate and export rate in the same units, mg CO_2 m^{-2} h^{-1} was converted to mg CH_2O by multiplying 0.68, the molar ratio of the two forms of carbon.

4.2.4 SPS extraction and assay

SPS enzyme extraction and assay were according to Huber and Huber (1991) as described in chapter 2.

4.2.5 ^{14}C labelling and determination

A ^{14}C labelling experiment was performed in order to determine carbohydrate transport rate. Six replicate shoots from each cultivar were used in this experiment. Whole side branch containing 10-15 lateral end branches was excised using a razor blade at 1000 h. The cut end was immediately immersed in a small vial containing 20

m*M* NaEDTA (pH 7.0) to prevent callose formation (King and Zeevaart 1974). The excised cut material was then brought into the laboratory where the stem was re-cut at the base under water to eliminate any trapped air in the xylem. The cut end was then immersed in a vial containing fresh EDTA solution and transferred into a growth chamber. After a 0.5 h equilibration period under an illumination of 400 μmol m^{-2} s^{-1} in a controlled environment room at 20°C, the shoots were enclosed in a plastic bag and photosynthetically labelled with a specific activity of 2 MBq (54 μCi) ^{14}C ($Na^{14}CO_3$). The method of feeding ^{14}C to plants was according to that of Flora and Madore (1996). ^{14}C was released by adding an adequate amount of lactic acid with a syringe into a beaker containing $Na^{14}CO_3$ solution which was suspended in the bag. After 20 min (1130 to 1150 h) of exposure of the fern to^{14}C the bag was removed. The shoot was re-cut again under water to a standard length of 2 cm from the branch nearest the cut end. The cut end was then immersed in a microfuge tube containing 2 mL of fresh 20 m*M* NaEDTA for collection of radiolabelled phloem exudate. The amount of ^{14}C exuded by the labelled ferns was determined, at approximately hourly intervals after the transfer, by removing 50 μL samples of the NaEDTA solutions for scintillation counting. Exudation was monitored for 9 hours. At the end of the experiment, the whole labelled fern was harvested to get fresh and dry weight data. Radioactivity in the exudute collected was measured in a liquid scintillation counter (Packard 2500 TR; Canberra-Packard, Dreieich, Germany) after addition of 2 mL of scintillation cocktail (Aquasafe 300 plus; Zinsser Analytic, Frankfurt, Germany) to the 50 μL exudate sample.

4.2.6 Statistical analysis

One way analysis of variance (ANOVA) was used to test for difference between the two cultivars. Differences were considered significant if $P < 0.05$. Regression analysis was performed to examine the relationship between carbon assimilation, sucrose concentration and carbon export.

4.3 Results

4.3.1 Diel changes in A and assimilate export

Fig. 4.1 shows the diel profile of *A* and assimilate export rate measured in mature cladophyll tissue. The patterns of *A* generally paralleled changes in light intensity during the light period. *A* increased rapidly in the morning, reached a maximum of 581 ± 44 (mg CH_2O m^{-2} h^{-1}) in ASP-69 and 405 ± 24 (mg CH_2O m^{-2} h^{-1}) in ASP-03 at about noon and then declined in the afternoon. ASP-69 displayed a significantly greater *A* than ASP-03 (Table 4.1). The mean rate of carbon fixation during the day period was 502 ± 53 (mg CH_2O m^{-2} h^{-1}) for ASP-69 and 356 ± 42 (mg CH_2O m^{-2} h^{-1}) for ASP-03, respectively.

The fluctuations in carbon export during the photoperiod closely reflected changes in photosynthesis (Fig. 4.1). Assimilate export increased in the morning, reached a maximum of 437 ± 42 CH_2O (mg m^{-2} h^{-1}) in ASP-69 and 319 ± 22 CH_2O (mg m^{-2} h^{-1}) in ASP-03 at about noon and then declined in the afternoon. Of net carbon assimilated at midday, approximately 75% was exported immediately in ASP-69, whereas 79% was exported in ASP-03 (Fig. 4.1). The mean carbon export rate during the light period was significantly greater in ASP-69 than in ASP-03 (Table 4.1). Toward the end of the light period, assimilate export rate exceeded the rate of carbon assimilation and was associated with depletion of cladophyll sucrose concentration (Table 4.1). Assimilate export remained fairly constant throughout the night (Fig. 4.1) at an average of 212 ± 31 CH_2O (mg m^{-2} h^{-1}) in ASP-69 and 112 ± 27 CH_2O (mg m^{-2} h^{-1}) in ASP-03 (Table 4.1) and was apparently linked to the mobilization of cladophyll sucrose (Fig. 4.2b). Similar to the light period, the mean carbon export rate during dark period was significantly greater in ASP-69 than in ASP-03 (Table 4.1). Respiratory loss of carbon at night was low and did not differ significantly between the two cultivars (Fig. 4.1)

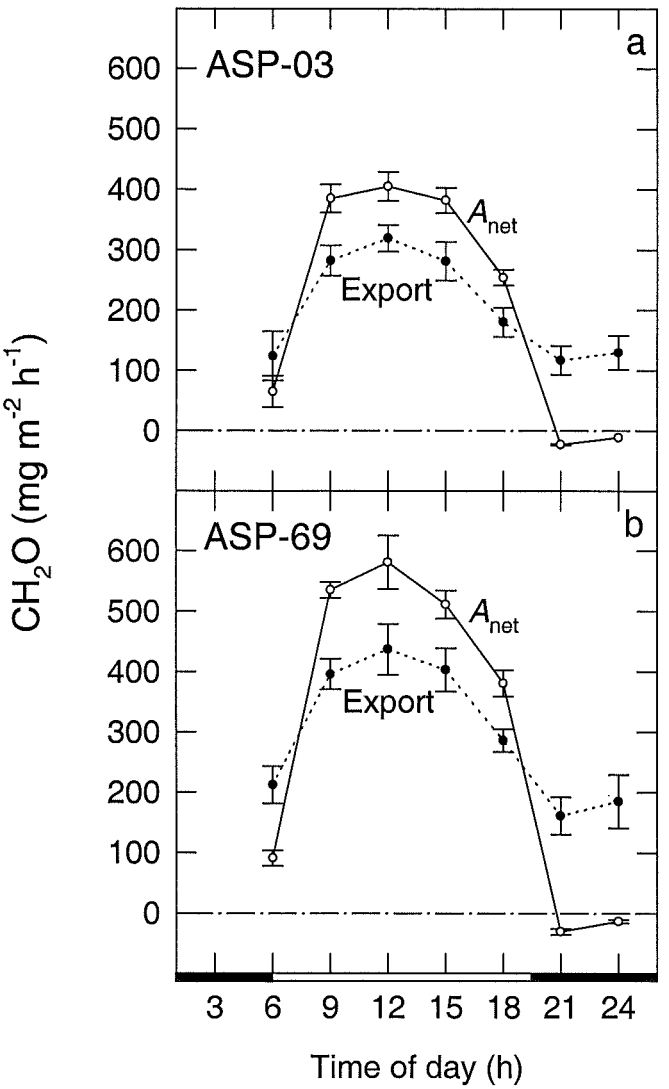


Fig. 4.1. Diel patterns of *A* and assimilate export rate in mature cladophyll tissue from two asparagus cultivars grown in the field. Results are means of 3 replicates ± SE for *A* and 6 replicates ± SE for assimilate export rate. The closed bar at the bottom of the figure indicates night and the open bar, day.

	Mean Assimilation rate ($mg\ m^{-2}\ h^{-1}$)	Mean Carbon export rate ($mg\ m^{-2}\ h^{-1}$)	Mean Sucrose concentration ($mg\ m^{-2}$)	Mean Starch concentration ($mg\ m^{-2}$)
Day				
ASP-69	502 ± 53	432 ± 57	3610 ± 240	1084 ± 148
ASP-03	356 ± 42	267 ± 45	2830 ± 159	903 ± 101
ANOVA	***	***	***	*
Night				
ASP-69	–	212 ± 31	2360 ± 215	946 ± 143
ASP-03	–	112 ± 27	2100 ± 211	781 ± 186
ANOVA	–	***	ns	ns

Table 4.1. Mean day and night carbon assimilation rate, export rate and carbohydrate levels in mature cladophyll tissue of two asparagus cultivars grown in the field. Results are mean values of individual measurements over day and night periods ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated as: ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 4.3 shows the relationship between *A* and export rate during the photoperiod (0600 to 1800 h) by plotting *A* against the rate of export calculated as the difference

between *A* and rate of accumulation in dry matter. A strong linear relationship between *A* and export rate (r = 0.86) was found by using all values obtained, indicating the greater the rate of photosynthesis and the greater the rate of carbon export. The greater assimilate export rate in ASP-69 was clearly correlated with a significantly higher carbon fixation rate in comparison to ASP-03 (Fig. 4.3).

4.3.2 Diel changes in carbohydrate concentration

Diel fluctuations in the concentrations of various sugars are shown in Fig. 4.2. The two cultivars displayed similar patterns of change of carbohydrate concentration. TNC per unit area increased throughout the light period, reaching a maximum of approximately 6500 mg m^{-2} for ASP-69 and 5200 mg m^{-2} for ASP-03 at the end of the day, respectively, and then declined with the onset of the dark period (Fig. 4.2a). Cladophyll TNC level in ASP-69 was greater than in ASP-03 ($P < 0.05$).

Sucrose was the principal component of the carbohydrate pool in cladophyll tissue, representing approximately 60% of total TNC. The changes in sucrose levels showed a similar pattern to those seen for TNC, increasing throughout the light period (Fig. 4.2b). Sucrose concentration reached a maximum of 3840 ± 150 mg m^{-2} for ASP-69 and 3130 ± 130 mg m^{-2} for ASP-03 in the late afternoon, and then decreased following the onset of the dark period. ASP-69 had a significantly greater sucrose concentration during the light period than did ASP-03 (Table 4.1). However, correlation between sucrose concentration and carbon export did not exist.

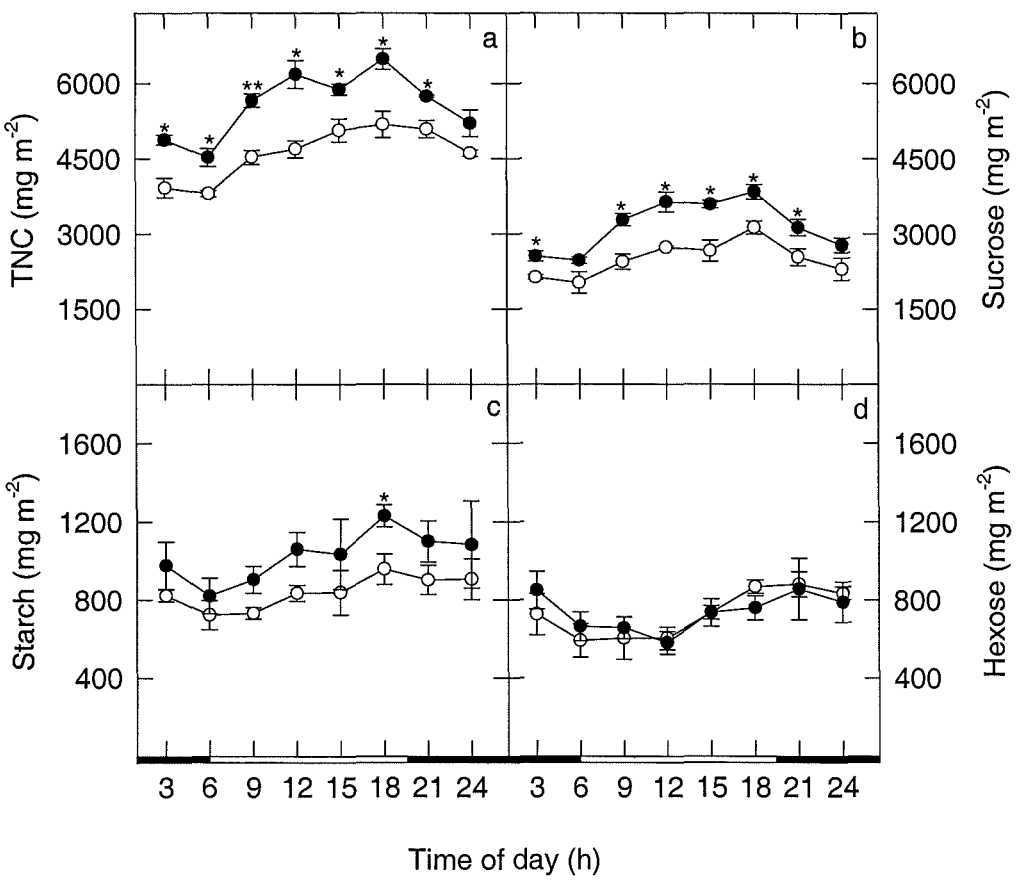


Fig. 4.2. Diel changes in (a) total non-structural carbohydrate (TNC), (b) sucrose, (c) starch and (d) hexose concentrations in mature cladophyll tissue from two asparagus cultivars (ASP-69, closed symbols; ASP-03, open symbols) grown in the field. Results are means of 3 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated as: * $P <$ 0.05, ** $P < 0.01$, *** $P < 0.001$.

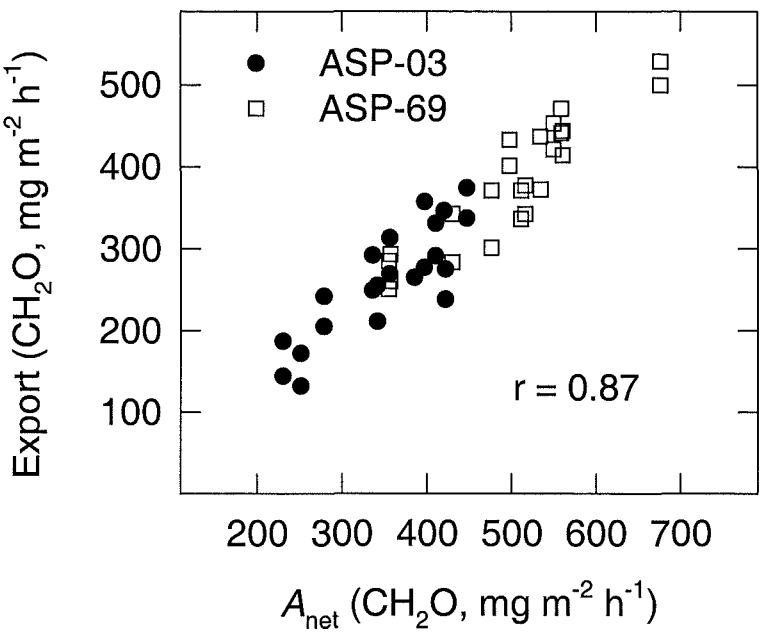


Fig. 4.3. Relationship between photosynthetic rate (*A*) and assimilate export rate during the photoperiod (0600 to 1800 h) by plotting rate of net photosynthesis against the rate of export calculated as the difference between rate of net photosynthesis and rate of accumulation in dry matter in two asparagus cultivars grown in the field.

In both cultivars, starch levels increased steadily during the day and then declined in the dark (Fig. 4.2c). ASP-69 displayed relatively higher starch concentrations than did ASP-03. Hexose concentrations decreased during the morning, followed by an increase toward the late afternoon, and then remained constant during dark period (Fig. 4.2d). There was no significant difference in hexose concentration between the two cultivars.

4.3.3 SPS activity

SPS activity showed no significant diel pattern, either in terms of substrate saturated (V_{max}) or substrate-limited (V_{lim}) activities (Table 4.2). Both V_{max} and V_{lim} in the dark period were similar to the activities observed in light period. Substrate-limited rates were approximately 60-65% of V_{max}. SPS activity was significantly higher in ASP-69 than ASP-03 during both day and night, but % activation was not different.

4.3.4 ^{14}C in phloem exudates

Analysis of phloem exudate showed that cladophyll tissue in both cultivars released appreciable amounts of photosynthetically fixed ^{14}C over the 9 h exudation period (Fig. 4.4a). The rate of carbon flux out of caldophyll tissue in ASP-69 calculated from the ^{14}C recovered in the phloem exudation was significantly greater than in ASP-03 (Fig. 4.4b). However, exudation patterns were very similar in both cultivars. Rate of ^{14}C exudation increased rapidly in the first three hours of chase period, reaching a maximum of 3.28 ± 0.34 (CPM 10^{-4}) g^{-1} FW h^{-1} in ASP-69 and 2.23 ± 0.38 (CPM 10^{-4}) g^{-1} FW h^{-1}. Exudation rate then remained constant for about an hour, after which it declined until end of the experiment.

	SPS activity		
	V_{max} (μmol m^{-2} h^{-1})	V_{lim} (μmol m^{-2} h^{-1})	Activation (V_{lim}/V_{max})
Day			
ASP-69	2000 ± 160	1260 ± 120	64.2 ± 3.0
ASP-03	1580 ± 70	920 ± 70	58.5 ± 3.4
ANOVA	***	*	ns
Night			
ASP-69	1950 ± 110	1200 ± 120	59.9 ± 3.9
ASP-03	1490 ± 60	920 ± 80	61.8 ± 4.7
ANOVA	**	*	ns

Table 4.2. Day and night SPS activity in mature cladophyll tissue of two asparagus cultivars grown in the field. Results are mean values of individual measurements over day and night periods ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated as: ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

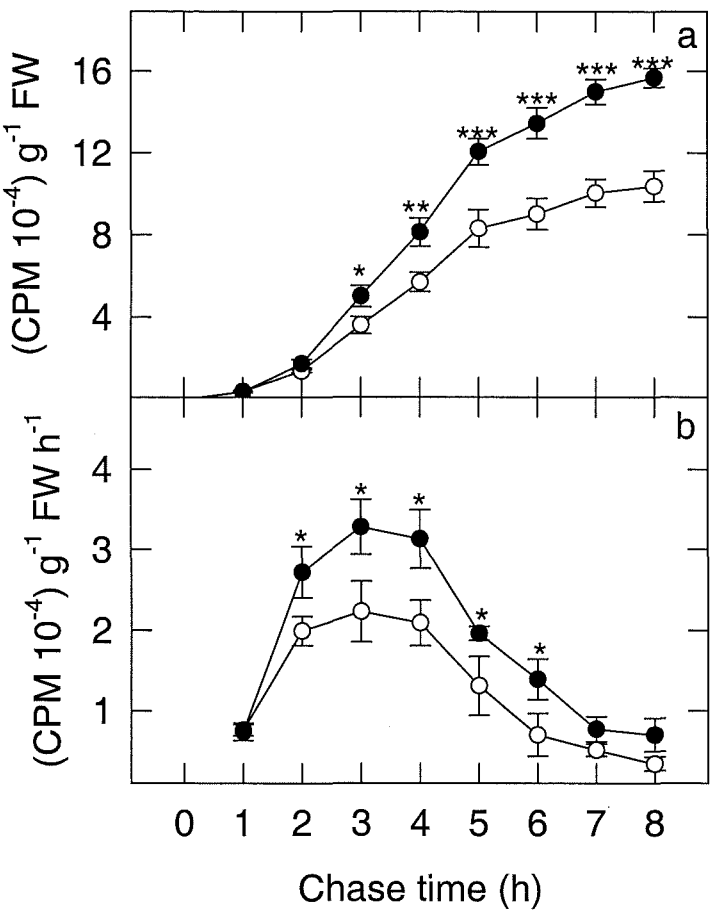


Fig. 4.4. Time-dependent exudation of photosynthetically fixed ^{14}C into NaEDTA solution from mature cladophylls of two asparagus cultivars (ASP-69, closed symbols; ASP-03, open symbols). (a) net increase of ^{14}C. (b) rate increase of ^{14}C. Results are means of 6 replicates ± SE. Cladophylls were pulsed for 20 min (1130 to 1150 h) with ^{14}C then chased with fresh NaEDTA solution for exudate collection. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated as: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4.4 Discussion

In mature photosynthetic tissue, net carbon gain during the photoperiod is allocated between leaf maintenance, storage and transport pools (Patrick 1988). The distribution of assimilates among these pools depends on carbon flux into sink tissue, which in turn is regulated by assimilation rate (feed-forward) and sink demand (feed-back), as well as environmental conditions (Daie 1985; Patrick 1988; Wardlaw 1990; Sinclair 1994). The findings in this chapter reveal a close relationship between photosynthetic rate and export rate in intact mature asparagus cladophyll tissue, consistent with the feed-forward relationship among photosynthesis, sucrose synthesis and immediate export. Both carbon fixation and partitioning influenced carbon export rate.

4.4.1 Sources of carbon for export

Sucrose and starch serve as sources of carbon for export or as a temporary reserve form of reduced carbon when carbon assimilation exceeds carbon export. Different species vary greatly in their sucrose/starch ratio. Species such as soybean (Chatterton and Silvius 1979) tend to accumulate primarily starch and little sucrose. In contrast, species that do not accumulate large amount of starch, such as barley (Gordon *et al.* 1980), tend to accumulate considerable concentrations of sucrose throughout the photoperiod. Accordingly, maintenance of carbon export on a daily cycle is dependent on a coordinated transition between the two carbon sources.

In the present study, sucrose was the principal carbohydrate for assimilate storage with minor storage of starch and hexose (Fig.4.2). Sucrose level increased during the light and decreased in the dark, indicating that sucrose is also the primary carbohydrate for export. This was particularly evident in the dark. Sucrose level declined rapidly with the onset of darkness (Fig. 4.2b), indicating that the rate of

carbon export in the dark is determined primarily by the sucrose accumulated during the light period. However, of the total sucrose storage pool, only approximately 30% was mobilized at night to support the dark growth, whereas 70% was retained in the cladophyll tissue (Fig. 4.2b). It has been shown that there are two types of sucrose pool in source tissues (Outlaw et al. 1975; Geiger et al. 1983). One pool exhibits high mobility (transport pool) and the other exhibits considerably lower mobility (storage pool) in terms of translocation out of source tissue. The vacuole is considered to be the transient storage pool with low mobility that acts as a buffer against short-term changes in photosynthesis and export from source tissue, whereas the cytoplasm is the transport pool with high mobility (Franceschi 1986). It is likely that the relatively low sucrose mobility at night was due to either a low sink demand caused by a low metabolic activity in sink tissue or a limited sucrose availability caused by the storage type in the source tissue.

In comparison to sucrose, degradation of starch was much slower except at the end of the dark period when sucrose decline slowed down (Fig. 4.2c). Thus, starch may contribute to carbon export when sucrose availability is limited. Therefore, it is concluded that, although both sucrose and starch were available for carbon export, sucrose was the preferred source of carbon for export. A possible reason for this could be that the high concentration of sucrose in the cladophyll tissue prevented the degradation of starch (Servaites et al. 1989; Galtier et al. 1993).

4.4.2 Sucrose partitioning and export

The partitioning of sucrose, the major transported form of fixed carbon, from photosynthetic tissue to growing or storage tissues, is a central physiological process regulating plant performance and agricultural yield (Wardlaw 1990). It is generally accepted that assimilate flow within the phloem is driven by a pressure difference generated by loading of osmotically active carbohydrates within the source and their

unloading in the sink (Gifford and Evans 1981; Madore 1995; Thorpe and Minchin 1996; Turgeon 2000). At the loading site, active sucrose transport is involved (Komor 2000). Consequently, carbon export rate should depend on sucrose availability in the leaves (Michaelis-Menten-type kinetics) and utilization in the sink tissue (Thorpe and Minchin 1996; Komor 2000). There is substantial evidence detailing a linear dependence of export rate on sucrose concentration (Fader and Koller 1983; Grodzinski *et al.* 1998). However, sucrose may also accumulate in response to environmental changes rather than as a result of excess for export (Huber et al. 1992; Daie 1996). For example, water stressed leaves usually partition more carbon into sucrose (Quick et al. 1992). In this case, a shift in allocation to favour sucrose accumulation may alter the availability of sucrose for export. In this study, sucrose concentrations increased continuously through the light period even though photosynthesis rate declined in the afternoon (Fig. 4.2b). The substantial increase in the sucrose pool in the afternoon may have been due to the fact that assimilation exceeded export and carbon accumulated in the cladophyll tissue or it may have been a response to environmental conditions experienced by the plants. In some species, significant effects on the partitioning of photosynthates in response to osmotic stress caused by an extended light period or water stress have been observed (Hanson and Hitz 1982; Weimberg *et al.* 1982). Undoubtedly, a shift in partitioning to sucrose accumulation in the vacuole of cladophyll cells would favour osmoregulation under water stressed conditions. It is possible that the accumulation of sucrose in the afternoon, in addition to its importance as a reserve to maintain export in the following dark period, may also provide a buffer against changing water status under field conditions. The fact that asparagus is a highly temperature dependent plant species which grows faster during the day than at night (Robb 1984), and that active storage root development occurs in the fern growing season (Pressman *et al.* 1993) suggests that it is unlikely that the increased sucrose accumulation in the afternoon is due to a low sink demand.

4.4.3 Cultivar variation in carbon assimilation, partitioning and export

The relationship between photosynthesis and crop yield is complex as crop plants consist of multiple sources and sinks, and agricultural yield is not necessarily synonymous with biological yield. Apart from assimilate production, efficiently partitioning a high proportion of those assimilates into economically important organs is a major determinant of crop yield (Daie 1985). However, agricultural yield in asparagus may be considered as synonymous with biomass production as the harvestable yield is actively growing shoots. Thus, even in the absence of allocation of carbohydrate among various sinks in asparagus, a close relationship between assimilation and asparagus yield might be expected. Indeed, greater assimilation rate in ASP-69 than in ASP-03 was found to be associated with greater yield. However, allocation of translocated assimilates is primarily determined by sink demand rather than source supply (Daie 1985). Therefore, further investigations into whole plant allocation patterns are needed to establish the link between carbon assimilation and yield in this crop species.

Comparison of carbon assimilation and export in the two cultivars showed that greater carbon export rates during the light period in high-yielding cultivar ASP-69 were associated with a greater rates of carbon fixation in comparison to the low-yielding cultivar ASP-03 (Fig. 4.3). Similarly, ASP-69 also accumulated considerably more sucrose in cladophyll tissue during the day than did ASP-03 (Fig. 4.2b). However, the correlation between sucrose concentration and carbon export did not exist and when the average values for export rate during light period were expressed as a percentage of assimilation rates, both cultivars displayed similar export capacities (Fig. 4.1). This suggests that the controls in initial partitioning of assimilates to immediate export or temporary storage pools within the cladophyll are similar in the two cultivars.

Analysis of the relationship between photosynthesis and carbon export indicates that higher photosynthetic rate in ASP-69 was associated with a greater carbon export rate in comparison with ASP-03 (Table 4.1). Moreover, ASP-69 revealed a greater SPS activity than in ASP-03. These data support a feed-forward relationship among photosynthesis, sucrose synthesis and assimilate export (Stitt et al. 1987; Geiger and Servaites 1994). The greater sucrose availability in ASP-69 was correlated with a greater SPS activity, which is a function of carbon fixation rate (Huber 1989). Lack of SPS activation by light in both cultivars suggests that regulation of asparagus SPS activity may be more similar to that of group III species (soybean, pea, tobacco) categorized by Huber et al. (1989). In both group I and II species, such as maize, barley and spinach, SPS activity is sensitive to light activation, whereas in group III species SPS does not respond to light activation. Comparison of diel changes in carbon assimilation, partitioning and export in mature asparagus cladophyll tissue between the two cultivars indicates the importance of carbon assimilation and sucrose synthesis in maintaining export rate during the day/night cycle. A balance in sucrose synthesis, storage and export during the light period provides a consistent carbon supply to sinks (i.e. the storage root) in the dark period. Phloem ^{14}C exudation confirmed the results obtained by mass changes and indicated that greater assimilation rate in ASP-69 may directly lead to a greater ^{14}C flux out of cladophyll tissue in comparison to ASP-03. It is concluded that there is a strong relationship between photosynthesis and assimilate export rate in the two asparagus cultivars investigated here. These data are consistent with a feed-forward relationship among photosynthesis, sucrose synthesis and assimilate export.

4.5 Summary

To assess the physiological aspects underpinning cultivar difference in asparagus (*Asparagus officinalis* L.) yield, diel photosynthetic rate (*A*), assimilate export and partitioning and sucrose phosphate synthase (SPS) activity were examined in mature cladophylls of two asparagus cultivars (ASP-69 and ASP-03) under field conditions.

Both cultivars exhibited similar diel patterns in *A* and carbohydrate partitioning. Rates of assimilate export estimated from *A* and dry mass changes were highest at midday and coincided with maximum assimilation rate. Assimilate export rate accounted for about 75% of carbon fixation during the photoperiod and was relatively constant at night, consistent with a decline in sucrose concentration. No diel fluctuations were observed in SPS activity in either cultivar. Sucrose was the principal carbohydrate for export in both cultivars, with levels in cladophyll tissue increasing during the day and decreasing in the dark. A positive correlation was found between *A* and assimilate export rate ($r = 0.87$) and this relationship did not differ between the two cultivars studied. The greater carbon export rate per unit area measured in the high-yielding cultivar (ASP-69) was associated with significantly higher *A* in comparison to the low-yielding cultivar (ASP-03). However, the correlation between sucrose concentration and carbon export rate did not exist. Biochemical evidence indicated that the greater *A* in ASP-69 was associated with a significantly higher SPS activity ($P < 0.05$). Phloem ^{14}C exudate analysis confirmed the results estimated by dry mass changes and revealed that ^{14}C flux out of cladophyll tissue in ASP-69 was significantly greater than in ASP-03. These results are consistent with a feed-forward relationship among photosynthesis, sucrose synthesis and assimilate export, indicating that differences in carbon fixation and sucrose synthesis in the two cultivars have a direct influence on carbon export.

While carbon assimilation and partitioning are positively linked to spear yield in the two cultivars, variation in sucrose metabolism in the developing spears may also contribute to cultivar difference in spear yield. Experiments described in chapter 5 will address this topic.

CHAPTER 5

CARBON METABOLISM IN DEVELOPING SPEARS

Chapter 5

Carbon Metabolism in Developing Spears

5.1 Introduction

The preceding chapters have indicated that carbon assimilation and its partitioning into storage roots are closely coupled in the two cultivars studied and greater photosynthetic rate is associated with a greater carbohydrate storage in the storage roots. This indicates that photosynthesis has a direct influence on carbon export and its allocation into storage root tissue. In this context, it should be expected that a close relationship exists between the amount of storage carbohydrate and spear yield. Indeed, high-yielding cultivars generally possess a greater carbohydrate storage pool in comparison to low-yielding cultivars (Benson and Takatori, 1980; also in Chapter 3). However, it is important to note that only a small amount of storage carbohydrate is utilised during spear development (Pressman *et al.* 1993; Faville *et al.* 1999a). The post-harvest development of the substantial shoot biomass appears a more severe drain on root carbohydrate (Faville, 1997; Woolley *et al.* 1999). Thus, processes contributing to overall yield in asparagus are highly integrated and involve not only the maintenance of high levels of carbon assimilation but also the efficient allocation of carbon into storage roots and its re-allocation into developing spears. High-yielding asparagus cultivars are generally associated with large canopy size and underground root systems (Tiedjens 1924; Ellison *et al.* 1960; Benson and Takatori 1980; Dufault and Greig 1983). These results suggest that spear yield may depend not only on the

availability of storage carbohydrates (carbon supply) but also on the ability of developing spears to attract and utilise them (carbon demand).

Sucrose has been identified as the major carbohydrate translocated from storage roots to developing spears, where it is hydrolysed into hexose and utilised in growth (Hurst *et al.* 1993; Irving and Hurst 1993). As a consequence, sucrose-metabolising enzymes such as acid invertase (AI), sucrose synthase (SS) and neutral invertase (NI) may play a major role in cleaving imported sucrose, which in turn may regulate the rate of carbon import into developing sinks (Johnson *et al.* 1988; Farrar 1996). In some species, a positive correlation between the rate of growth and sucrose metabolism has been illustrated (Chanda *et al.* 1986; Sung *et al.* 1989; Wardlaw 1990; Black 1993; Jenner and Hawker 1993; Wang *et al.* 1993). This led to the assumption that sucrose-cleaving enzymes may be used as an indicator of sink ability in attracting carbohydrates from source tissue (Sung *et al.* 1994; Farrar 1996).

Progress has been made in recent years in understanding the regulation of carbon import into developing spears of asparagus. Based on metabolic status and accumulation of carbohydrates, the critical role of spear tips in controlling spear growth has been identified (King *et al.* 1990; Lill *et al.* 1990). However, the relative roles of sucrose cleaving enzymes in the determination of carbon import and their relationship with spear growth are still not fully understood. Hurst *et al.* (1993) reported that AI activity was 4 times greater in the middle of the spear than in the tips and bases, whereas SS and NI activities were similar throughout the spear. In contrast, Alam *et al.* (1999) found that SS activity was greater in the base than in the tip. In another similar study, Irving and Hurst (1993) observed that SS activity was much greater than AI in spear tips. Since the changes in activities of sucrose-cleaving enzymes may be correlated with sink functions, including import, storage reserve and biosynthetic activities in the developing tissue (Sung *et al.* 1994), the relative importance of sucrose cleaving enzymes in carbon metabolism may differ with

changes in sink metabolism. Thus, the patterns of carbon import into a growth tissue may be determined by various sink functions during the development of sink tissues.

The experiments described in this chapter were conducted to test the hypothesis that spear growth is limited by sink ability in attracting carbon from source tissue. The approach was to establish a relationship between spear growth and carbohydrate content as well as its metabolism in the developing spears. Carbon metabolism was examined in the early stages of spear growth during which the carbohydrate supply was considered to be at a maximum.

5.2 Materials and methods

5.2.1 Shoot growth rate

Shoot elongation rate (mm h^{-1}) and relative growth rate (RGR) were measured on a 24 h basis in two separate clear days (2^{nd} and 19^{th} October 1999) with similar conditions in order to cover the full range of shoot growth stages. The mean daily air temperature was 14.8°C and 13.7°C, respectively. The measurements in each growth stage were classified as follows: All values from shoots below 10 cm in height were put into stage 1, those between 10 and 20 cm were put into stage 2, and so on. To further establish patterns of spear elongation, spears of different height (6, 10, 15, 20 and 25 cm) were marked at 3 cm intervals from tip to base and the elongation rate for each section was recorded during 0830 to 1830 h on the measuring day. Elongation rate was calculated as the average elongation rate increase per hour of the growth over the measuring period. RGR was calculated as

$RGR = (\ln H^2 - \ln H^1) / (t^2 - t^1)$

where H^2 and H^1 are spear height at time t^2 and t^1 respectively.

5.2.2 Tissue harvesting

Carbon metabolism investigations focused on young developing spears with heights up to 25 cm because: (1) During shoot growth measurements, it was observed that the short spear in ASP-03 usually branched earlier than ASP-69 (field observation), which might cause a shift in spear growth from extension to radial growth in the sub-apical region of the spear. (2) Carbohydrate supply was assumed to be under maximum conditions in young developing spears. This allowed carbon metabolism to be studied in similar conditions in the two cultivars. Spears of different height (5, 10, 15, 20 and 25 cm) were collected between 12:00 and 13:00 h on the sampling day. A section of 1.5 cm directly below the spear tip was immediately excised into three lengthwise segments. One segment was used for enzyme extraction, one segment was used for carbohydrate extraction, and the remaining segment was used to determine water content. The segments destined for carbohydrate and enzyme determinations were quickly weighed and immediately frozen in liquid nitrogen and then stored at -80°C prior to assay, whereas the segments for water content determination were immediately weighed and then freeze-dried to obtain dry weight (DW). For the spear section studies, market-sized spears at a height of about 25 cm were sampled, quickly cut into five 5 cm sections from tip to base, and a portion of 1.5 cm counted from base of each section was excised again and then processed as above.

5.2.3 Carbohydrate analysis

Total non-structural carbohydrate (TNC), soluble sugar and starch content were determined using the methanol:chloroform:distilled water method of Tissue and

Wright (1995) as described in chapter 2. Sucrose and Hexose sugars were determined according to William *et al.* (1988) as described in chapter 2.

5.2.4 Enzyme extraction and assay

To investigate sucrose metabolism in spear tissues, activities of the sucrose-cleaving enzymes AI, NI and SS were measured in the spear elongation zones at different stages of development and in different sections of market-sized spears (25 cm). Frozen tissue (1g FW) was extracted by grinding in a pre-cooled mortar using 5 mL extraction buffer (pH 7.5) containing 50 mM HEPES, 50 mM $MgCl_2$, 10 mM EDTA, 10 mM EGTA, 2.5 mM DTT and 0.1% Triton X-100. The homogenates were centrifuged at 4°C and 11,000 × *g* for 5 min in 10 mL centrifuge tubes. Extract of 1 mL was collected for quantification of total soluble proteins using the Bio-Rad Coomassie blue protein assay (Bio-Rad, München) against a protein (BSA) standard (Bradford, 1976). The remaining supernatant was immediately desalted on Sephadex G-25 (Sigma Chemical Co.) columns equilibrated with grind buffer minus the Triton X-100. AI, NI and SS activities were assayed immediately after desalting as described in chapter 3.

5.2.5 Statistical analysis

All growth data presented are the means ± SE of 6 replicates, whereas spear section elongation and enzymatic data are the means ± SE of 4 replicates. One way analysis of variance (anova) was used to test for difference between the two cultivars. Differences were considered significant if $P < 0.05$.

5.3 Results

5.3.1 Shoot growth

Shoot growth data measured during different growth stages are presented in Fig. 5.1 and Table 5.1. Elongation rate of the whole shoot on a 24 h basis in ASP-69 was significantly higher ($P < 0.05$) than in ASP-03 for nearly all growing stages (Fig. 5.1*a*). Growth patterns, however, were very similar in both cultivars. Upon emergence, elongation rate increased rapidly, reaching a maximum of 5.4 mm h^{-1} for ASP-69 at spear height about 40 cm and 3.2 mm h^{-1} for ASP-03 at height about 30 cm. Elongation rate then remained constant to a height of about 90 cm for ASP-69 and 70 cm for ASP-03, after which it declined until reaching the final height. After about 5 weeks, the growing fern reached an average final height of about 175 cm for ASP-69 and about 120 cm for ASP-03 respectively (data not shown). Elongation data for spear sections (Table 5.1) showed that in both cultivars, the spear tip had a greater elongation rate than the middle and basal sections in early stages of development (6 and 10 cm. Table 5.1). Then, the zone of maximum elongation shifted towards the middle of the spear with further increases in spear height (15, 20 and 25 cm. Table 5.1). It was noted that the length of the elongation zone increased with the height of spear and was greater in ASP-69 than in ASP-03 (Table 5.1). For example, at spear height of 10 cm, the elongation zone in both cultivars was approximately 6 cm in length from tip towards base and the maximum rate of elongation was in the section 1. When spear height reached 25 cm, the elongation zone in ASP-69 increased to approximately 12 cm in length and the maximum rate of elongation was shifted to section 3, whereas in ASP-03 the elongation zone was approximately 9 cm in length and the maximum rate of elongation was shifted to section 2. Similarly, the maximum rate of elongation was also greater in ASP-69 than in ASP-03. However, The maximum elongation rate per unit length (3 cm) for each cultivar was relatively constant in comparison to the changes in length of elongation zone with the further increase in height in each cultivar (Table 5.1). It appeared that as spear developed, the

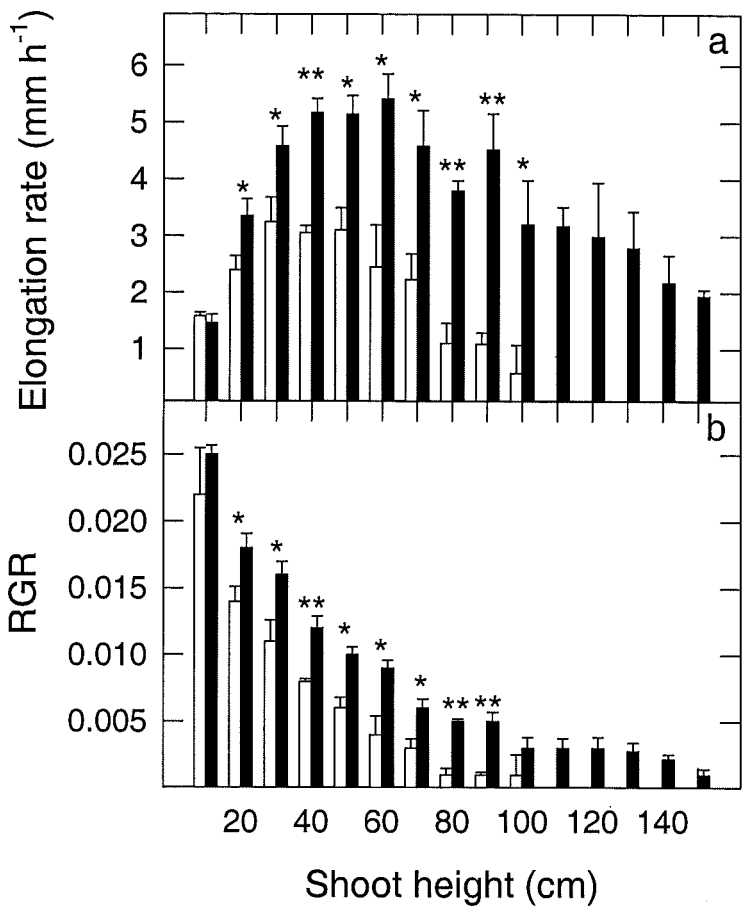


Fig. 5.1. Shoot elongation rate (a) and RGR (b) on a 24 h basis over the whole range of growth stages in high-yielding cultivar ASP-69 (closed bar) and low-yielding cultivar ASP-03 (open bar). Results are measurements of 6 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated * $P < 0.05$, ** $P < 0.01$.

increase in elongation rate of whole spear was mainly the result of an increase in the size of the elongation zone rather than an increase in the rate of elongation. Relative growth rate (RGR) of the whole spear declined with increasing height in both cultivars, and was also significantly greater in ASP-69 than in ASP-03 (Fig. 5.1*b*).

Water content of spear tips remained constant throughout spear developmental stages and there was no significant difference between the two cultivars (Table 5.2). In the elongation zone, water content increased initially with age in both cultivars and then remained constant with a mean value of 10.3 ± 0.46 g H_2O g^{-1} DW in ASP-69 and 9.9 ± 0.38 g H_2O g^{-1} DW in ASP-03 after spear height reached 15 cm. Along the sections of market-sized spears, water content increased from tip to the elongation zone, reaching a maximum of 12.0 ± 0.66 g H_2O g^{-1} DW in ASP-69 and 10.3 ± 0.54 g H_2O g^{-1} DW in ASP-03 and then declined in the base section. There was no significant difference between the two cultivars either in elongation zones with age or along the sections of market-sized spears. Protein content in the elongation zone was essentially similar with age (227 ± 12 mg g^{-1} DW in ASP-69 and 218 ± 13 mg g^{-1} DW) and there was no significant difference between the two cultivars. In the spear sections, protein content was highest in the spear tip (316 ± 9 mg g^{-1} DW in ASP-69 and 300 ± 9 mg g^{-1} DW in ASP-03) and decreased consistently from tip to base (109 ± 4 mg g^{-1} DW in ASP-69 and 112 ± 8 mg g^{-1} DW in ASP-03), and again there was no significant difference between the two cultivars.

Spear height	Cultivar	Elongation rate (mm h^{-1}) Spear section					
		1	2	3	4	5	6
6 cm	ASP-03	0.40 ± 0.11	0.36 ± 0.14				
	ASP-69	0.44 ± 0.03	0.37 ± 0.16				
	ANOVA	ns	ns				
10 cm	ASP-03	0.75 ± 0.21	0.62 ± 0.39	0.08 ± 0.04			
	ASP-69	0.99 ± 0.25	0.97 ± 0.20	0.29 ± 0.07			
	ANOVA	ns	ns	***			
15 cm	ASP-03	0.81 ± 0.16	0.97 ± 0.08	0.39 ± 0.11	0.04 ± 0.03	0.01 ± 0.00	
	ASP-69	0.92 ± 0.11	1.11 ± 0.23	1.06 ± 0.24	0.31 ± 0.03	0.03 ± 0.03	
	ANOVA	ns	ns	***	***	ns	
20 cm	ASP-03	0.82 ± 0.06	0.97 ± 0.10	0.71 ± 0.11	0.14 ± 0.05	0.03 ± 0.02	0
	ASP-69	0.81 ± 0.14	1.00 ± 0.13	1.23 ± 0.10	0.86 ± 0.09	0.30 ± 0.09	0.06 ± 0.02
	ANOVA	ns	ns	***	***	***	**
25 cm	ASP-03	0.65 ± 0.17	0.86 ± 0.11	0.48 ± 0.14	0.03 ± 0.02	0	0
	ASP-69	0.76 ± 0.08	1.04 ± 0.12	1.34 ± 0.12	1.02 ± 0.15	0.16 ± 0.08	0.01 ± 0.01
	ANOVA	ns	*	***	***	**	ns

Table 5.1. Elongation rate (mm h^{-1}) in different spear sections of developing spears in high-yielding cultivar ASP-69 and low-yielding cultivar ASP-03. 1, 2, 3, 4, 5 and 6 indicate sections of spears at 3 cm intervals from tip toward base at spear heights of 6 cm, 10 cm, 15, cm, 20 cm and 25 cm. Results are means of 6 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Water content (g H_2O g^{-1} DW)			**Protein content** (mg g^{-1} DW)		
	ASP-03	ASP-69	ANOVA	ASP-03	ASP-69	ANOVA
Elongation zone						
I	8.38 ± 0.18	8.97 ± 0.40	ns	224 ± 15.3	214 ± 8.5	ns
II	8.79 ± 0.08	8.78 ± 0.04	ns	227 ± 15.5	249 ± 10.5	ns
III	10.00 ± 0.27	9.22 ± 0.47	ns	223 ± 9.9	214 ± 16.2	ns
IV	9.77 ± 0.22	10.73 ± 0.28	*	216 ± 17.5	235 ± 15.1	ns
V	9.81 ± 0.65	10.96 ± 0.64	ns	204 ± 5.2	223 ± 11.4	ns
Spear section						
Tip	7.31 ± 0.13	7.60 ± 0.08	ns	300 ± 9.4	316 ± 9.1	ns
5	8.47 ± 0.34	9.96 ± 0.41	*	246 ± 5.0	255 ± 7.3	ns
10	9.61 ± 0.86	10.76 ± 0.31	ns	217 ± 12.1	209 ± 12.5	ns
15	10.30 ± 0.54	12.00 ± 0.66	ns	156 ± 12.0	164 ± 15.4	ns
20	9.08 ± 0.17	11.57 ± 1.98	ns	109 ± 7.3	124 ± 14.7	ns
25	8.26 ± 0.56	9.97 ± 0.48	ns	112 ± 7.6	109 ± 4.4	ns

Table 5.2. Water content (g H_2O g^{-1} DW) and total soluble protein content (mg g^{-1} dw) in the elongation zone of developing spears (I, II, III, IV and V indicate stages of spear development at heights of 5 cm, 10 cm, 15, cm, 20 cm and 25 cm, respectively) and mature spear sections (tip, 5, 10, 15 20 and 25 cm from tip to base) in high-yielding cultivar ASP-69 and low-yielding cultivar ASP-03. Results are means of 3 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated as ns not significant where $P > 0.05$, * $P < 0.05$, ** $P < 0.01$.

5.3.2 Tissue carbohydrate content

Total non-structural carbohydrate (TNC) content was examined in the elongation zones of developing spears and in different sections of market-sized spears (25 cm). TNC remained constant in the elongation region with age and was generally greater in ASP-69 than in ASP-03 (Fig. 5.2*a*). In the spear sections, TNC increased from tip to base and was significantly greater in ASP-69 than in ASP-03 (Fig. 5.2*b*). Carbohydrate composition was similar in the two cultivars, with a higher soluble sugar content and a much lower starch content. Starch level remained relatively constant from tip to base and there was no significant difference between the two cultivars (data not shown). The predominant soluble sugars were hexoses. Hexose content in the elongation region increased initially with age and remained constant after spear height reached 10 cm (Fig. 5.3*a*). Significant differences in the two cultivars were observed in most developmental stages. Sucrose content was similar in the elongation zones in the two cultivars throughout development (Fig. 5.3*b*). Sucrose/hexose ratio declined slightly with age and was generally higher in ASP-03 than in ASP-69 (Fig. 5.3*c*). In spear sections, hexose content increased from tip to base and was significantly higher in ASP-69 than in ASP-03 throughout the spear (Fig. 5.4*a*). Sucrose content did not differ significantly between the two cultivars, decreasing from tip to the middle region of spears and then increasing in the base (Fig. 5.4*b*). The ratio of sucrose to hexoses declined from tip to base, and did not differ significantly between the cultivars (Fig. 5.4*c*).

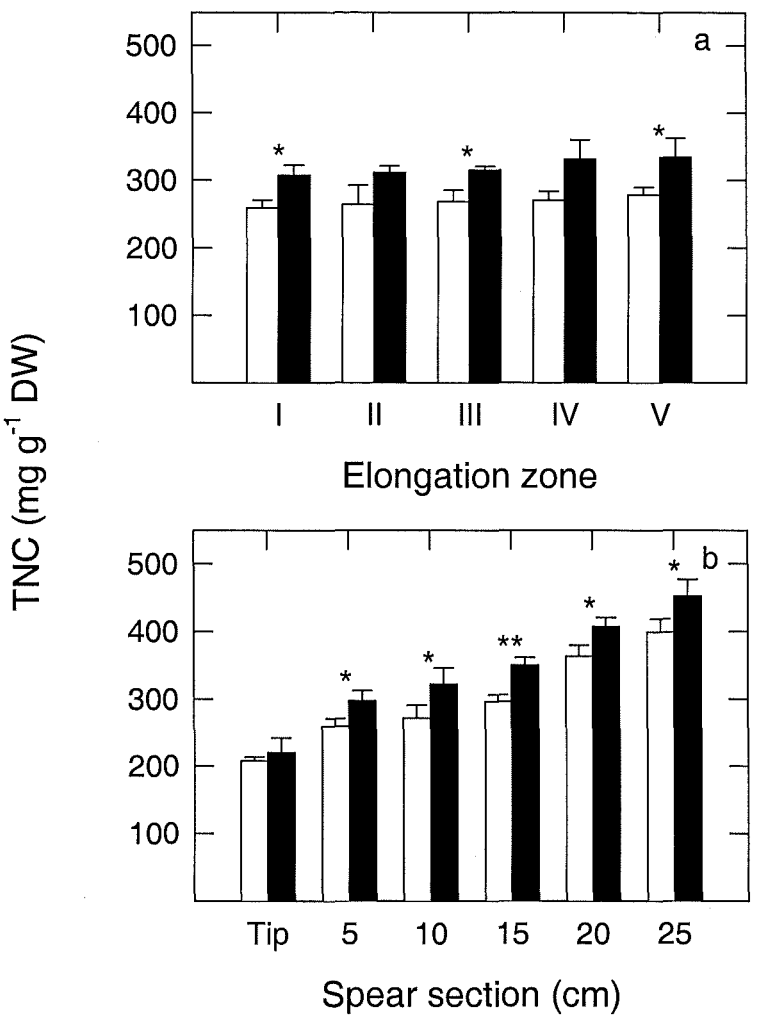


Fig. 5.2. Levels of TNC in the elongation zone of developing spears (a: I, II, III, IV and V indicate stages of spear development at heights of 5 cm, 10 cm, 15, cm, 20 cm and 25 cm) and in market-sized spear sections (b: tip, 5, 10, 15, 20 and 25 cm from tip to base) in ASP-69 (closed bar) and ASP-03 (open bar). Results are means of 3 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated * $P < 0.05$, ** $P < 0.01$.

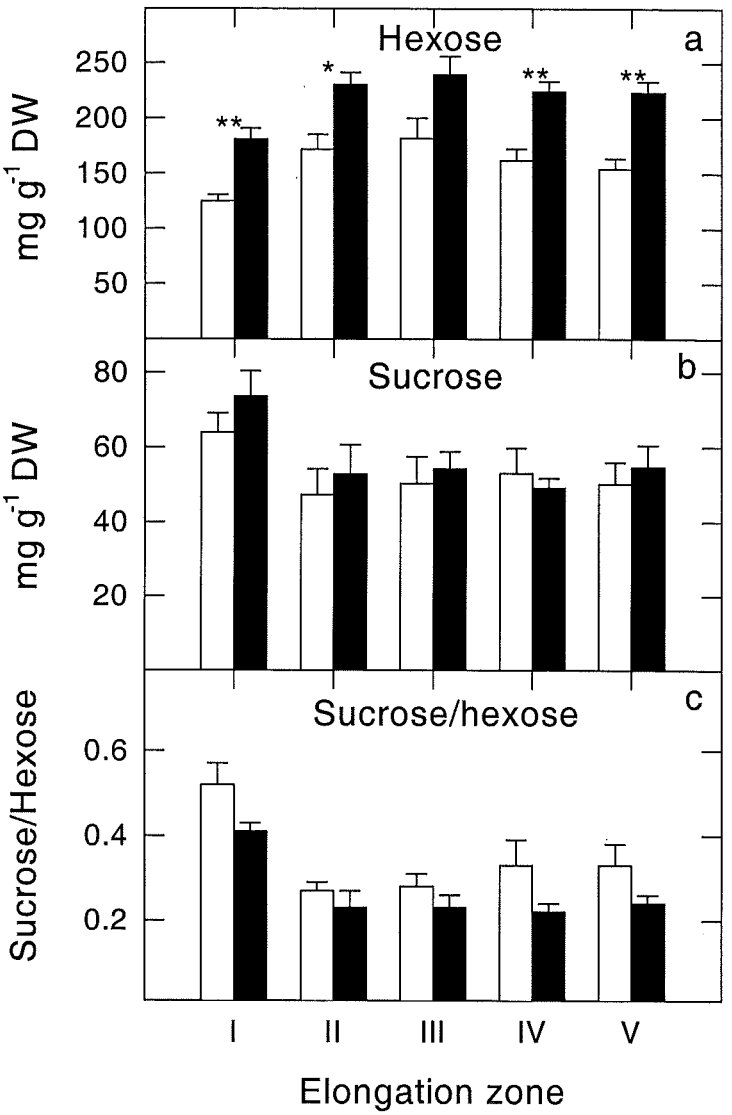


Fig. 5.3. Hexose (a) and sucrose (b) concentrations and sucrose/hexose ratio (c) in the elongation zone of developing spears in ASP-69 (closed bar) and ASP-03 (open bar). I, II, III, IV and V indicate stages of spear development at heights of 5 cm, 10 cm, 15, cm, 20 cm and 25 cm, respectively. Results are means of 3 replicates ± SE Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated * $P < 0.05$, ** $P < 0.01$.

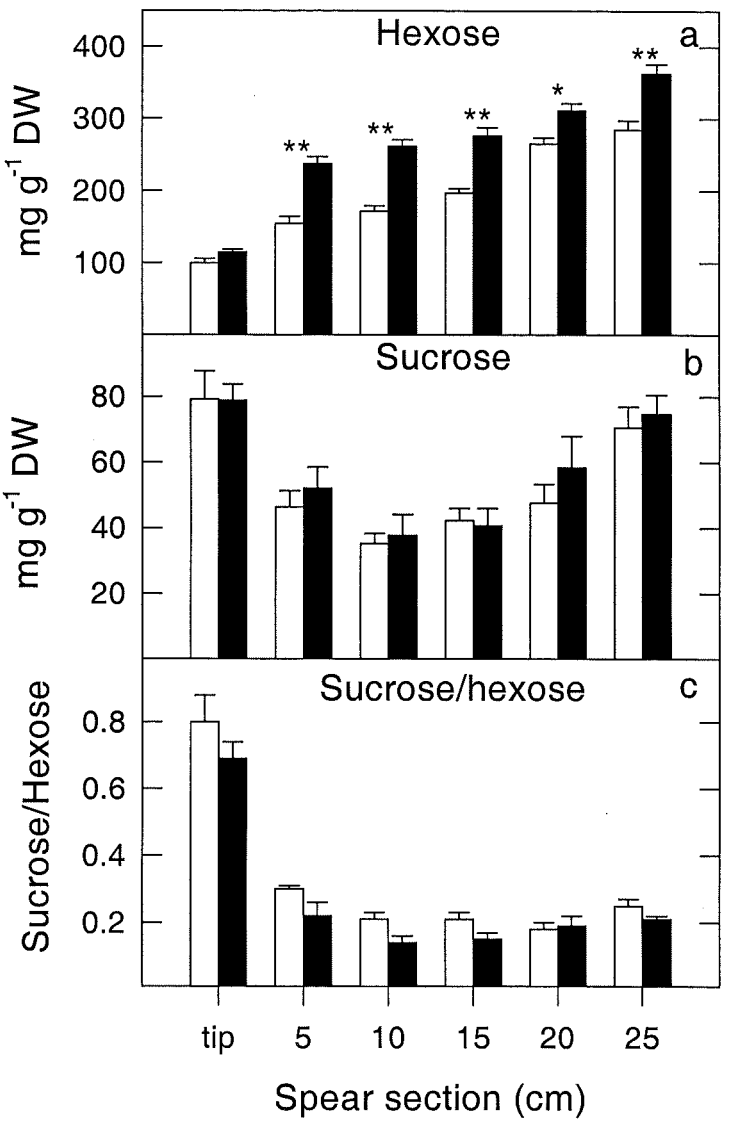


Fig. 5.4. Hexose (a) and sucrose (b) concentrations and sucrose/hexose ratio (c) in market-sized spear sections (tip, 5, 10, 15 20 and 25 cm from tip to base) in ASP-69 (closed bar) and ASP-03 (open bar). Results are means of 3 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated * $P < 0.05$, ** $P < 0.01$.

5.3.3 Enzyme activities

SS activity was very similar on a fresh weight basis in the spear elongation zone in the two cultivars, and remained constant throughout spear development (Fig. 5.5*a*). In contrast, AI activity changed progressively with developmental stage (Fig. 5.5*b*). Upon spear emergence, AI activity rapidly increased and reached maximum at a spear height of about 10 cm in ASP-03, and then remained constant with further development, whereas in ASP-69, AI activity appeared to increase consistently with age (Fig. 5.5*b*). The activity of AI in ASP-69 was significantly greater ($P < 0.05$) than in ASP-03 in all the developmental stages except at spear height of 5 cm. Activity of NI was rarely detectable in any regions of the spears (data not shown).

In spear sections, SS activity increased steadily along the length of the spear from tip to base in both cultivars, reaching a maximum in the base (Fig. 5.6*a*). SS activity was much lower than AI activity in the middle portion of spear from 5 to 20 cm, but in the tip and base portions, SS was the predominant sucrose-cleaving enzyme (Fig. 5.6*a*). There was no significant difference in SS activity between the two cultivars. In contrast, AI activity changed markedly along spear sections. From the tip to the middle of spears, AI activity rapidly increased, then declined toward the base section (Fig. 5.6*b*). AI activity was significantly greater in ASP-69 than in ASP-03 ($P < 0.05$), particularly in the middle portion of the spears.

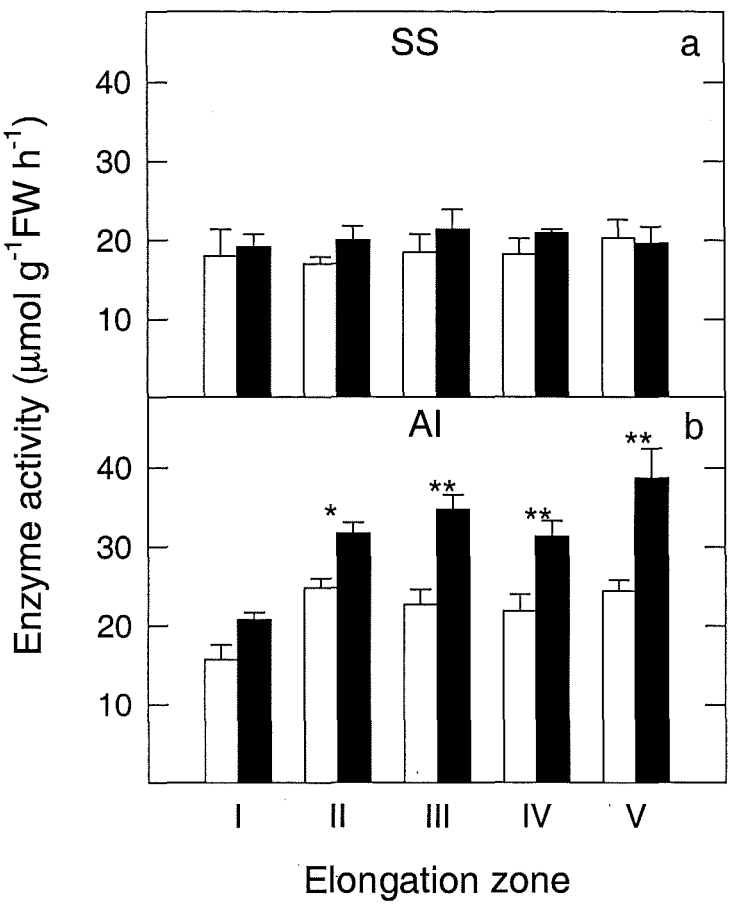


Fig. 5.5. Activities of SS (a) and AI (b) in the elongation zone of developing spears in ASP-69 (closed bar) and ASP-03 (open bar). I, II, III, IV and V indicate stages of spear development at heights of 5 cm, 10 cm, 15, cm, 20 cm and 25 cm, respectively. Enzyme activities are expressed as µmol sucrose produced h^{-1} g^{-1} FW for SS and µmol glucose produced h^{-1} g^{-1} FW for AI. Results are means of 3 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated * $P < 0.05$, ** $P < 0.01$.

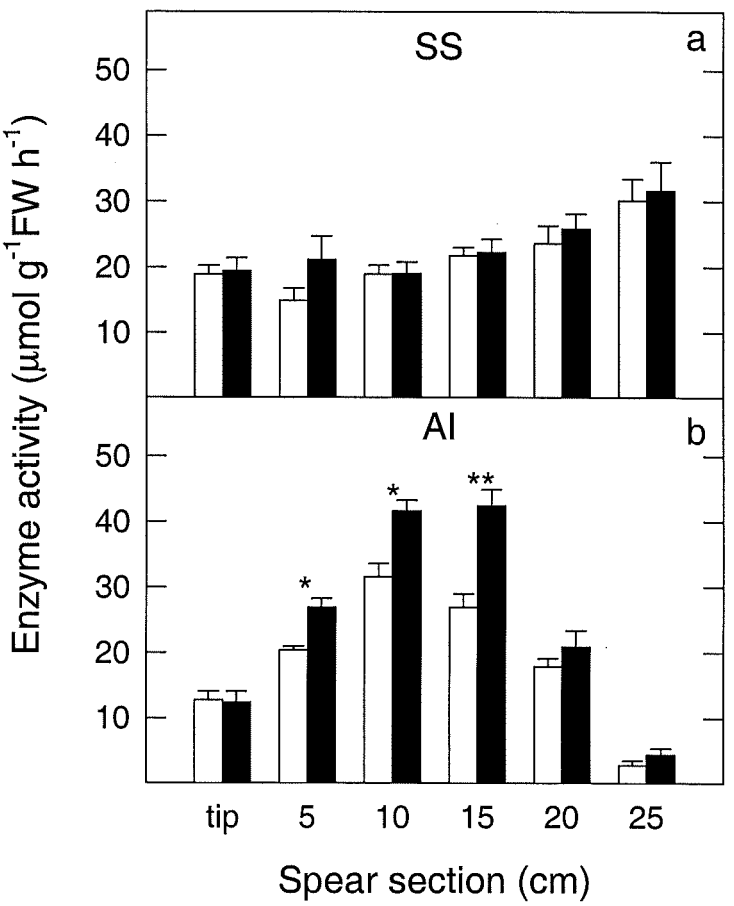


Fig. 5.6. Activities of SS (a) and AI (b) in market-sized spear sections (tip, 5, 10, 15 20 and 25 cm from tip to base) between ASP-69 (closed bar) and ASP-03 (open bar). Results are means of 3 replicates ± SE. Mean values are compared using one way ANOVA and significant differences between the two cultivars are indicated * $P <$ 0.05, ** $P < 0.01$.

5.4 Discussion

5.4.1 Cultivar variation in carbohydrate metabolism

The results obtained from this study indicate that higher growth rates in spears of ASP-69 are associated with a greater carbohydrate accumulation in spear tissue, particularly in the elongation and base regions of spears. This suggests that carbohydrate availability for developing spears is greater in ASP-69 than in ASP-03. However, measurements of individual carbohydrate components show that hexose content was greater in ASP-69 than in ASP-03, whereas sucrose content was not significantly different. This may indicate a more effective machinery for transport and catalysis of carbohydrate in spears of ASP-69 (Farrar 1996). Since the greater hexose accumulation in ASP-69 was not associated with a decrease in sucrose content compared to ASP-03, it is possible that the greater hexose accumulation in ASP-69 was due to greater activities of sucrose-cleaving enzymes rather than the availability of sucrose.

Measurements of enzyme activities revealed that the different utilisation of sucrose in the elongation zone of spears was mainly due to greater AI activity, not SS activity. This result is in agreement with that obtained by Hurst *et al.* (1993). In the present study, AI activity in the elongation zone of ASP-69 was generally greater than in ASP-03 except in the early growth stage, whereas SS activity was not significantly different throughout growth stages studied. There was little NI activity detected in either cultivar. These results suggest strongly that it is AI and not SS or NI that is an important determinant of the difference in sucrose metabolism in the two cultivars. As AI is confined to the cell wall or vacuole, whereas SS and NI are mainly located in the cytosol (King *et al.* 1997), these enzymes may have different roles in relation to carbon metabolism. High activity of AI may not only provide substrates and reducing

power for growth (King *et al.* 1997), but also assist in cell expansion and regulate carbon import by increasing osmotic potential by cleaving sucrose into two hexose moieties (Irving *et al.* 1997).

The ratio of sucrose to hexose has been used as a measure of sink activity during development (Einig *et al.* 1999). It is generally lower in growing, sugar importing sink tissue and higher when tissues mature as a result of changes in carbohydrate metabolism (Ross *et al.* 1994). In this study, sucrose/hexose ratio in the elongation region decreased considerably in early development in both cultivars and then remained constant with an increase in height, indicating a consistently active conversion from sucrose to hexose. Since the developing spears are still active growing tissues and dependent mainly on imported carbohydrate, it is expected that the shift from carbohydrate import (sink) to export (source), a typical transition occurring during development, does not occur during spear growth. In addition, carbohydrate availability is not considered to be a limiting factor during spear development (Pressman *et al.* 1993; Faville 1997; Woolley *et al.* 1999). Thus, hexose content, rather than sucrose content, may be used as an indication of sink activity in the elongation zone of developing spears.

5.4.2 Carbohydrate metabolism in specific spear regions

Although a significant difference in carbohydrate metabolism was observed between the two cultivars (Chapter 3), the patterns of carbohydrate content in the sections of mature spears were essentially similar. The highest hexose content was in the base, not in the elongation region of spears. There are several potential reasons for the lack of relationship between elongation and hexose accumulation. Firstly, unlike most crops, asparagus spears grow much faster during the day than at night (Robb 1984). A diluting effect in the expansion zone due to the rapid use of hexose for the formation of cellulose and other structural substances may account for the low concentration in

this region (Culpepper and Moon 1939b). Secondly, it is evident that lignification of shoot vascular tissue is a long-term process throughout shoot development. Culpepper (1939) recorded that lignification of vascular tissue in spear bases increased rapidly as spears increased in height, and the segments immediately above the base continued to lignify when cell elongation ceased. Thus, the extent of the lignifying portion of the spear may continue to increase upward as spears approach maturity. This process would require a consistent supply of hexose. Thirdly, it is possible that there is a biochemical inactivation in enzyme pools along the spear sections (Sung *et al.* 1994).

In some crops, different sucrose-cleaving enzymes and related pathways are associated with different biosynthetic or storage processes. For instance, Doehlert (1990) concluded that different parts of developing maize kernels differ in their enzyme composition, reflecting the differences in their accumulation of product. Sung *et al.* (1994) reported that the relative importance of AI and SS changes during the development of *Phaseolus vulgaris* fruit. In this study, although there is no clear correlation between enzyme activity and hexose accumulation, hexose content did increase in parallel with AI activity from the tip to the middle of the spear, but then continued to increase towards the base even though AI activity declined markedly. These results are consistent with those reported by Hurst *et al.* (1993) who suggested that the rate of hexose usage, rather than sucrose-cleaving enzyme activity, is the main determinant of hexose concentration in spears. In our results, the highest invertase activity was not in the region of maximum rate of elongation, but in the middle of the spears, where initial lignification of cell walls occurs before growth finally ceases (Culpepper and Moon 1939a). It is possible that invertase was not only a major enzyme for hexose formation in the cell expansion process, but was also involved in the cell wall lignification process. This may be a key process in providing hexose for both rapid elongation and initiation of cell wall thickening during the day.

Although SS activity did not appear responsible for cultivar differences in carbohydrate metabolism, there was a tendency for SS activity to be highest in the

base in both cultivars. This difference was clearly associated with carbohydrate accumulation along the spear. The importance of SS activity may be related to the storage phase of carbohydrate usage rather than its utilisation (Sonnewald and Willmitzer 1992; Sung *et al.* 1994). For instance, Sung *et al.* (1989) reported that total SS activity was related to sucrose import by a sucrose-accumulating sink. In this study, changes in TNC and hexose accumulations from tip to base were associated well with SS activity. These results substantiate the hypothesis that the major role of SS is related to accumulation of imported sugar rather than its utilisation in elongation growth.

5.4.3 Carbohydrate metabolism — a whole spear property

The concept of sink regulation in carbon partitioning has been used to explain the coordination of carbohydrate availability and its consumption in actively growing tissue (Stitt *et al.* 1990; Hajirezaei *et al.* 2000). It is hypothesized that the ability of a sink to maintain a high rate of utilisation of imported sugars, either by metabolism or compartmentation, should enhance its mobilising ability (Marcelis 1996). The profile of enzyme activity and carbohydrate accumulation reported here indicates that spear development involves both anatomical and physiological changes, leading to the differentiation of developing spears into three distinctly physiological units — tip, elongation zone and base.

Spear tips can be considered as a metabolic sink, utilising imported sucrose to provide energy for growth through respiration (Lill *et al.* 1990; Hurst *et al.* 1993). SS has been reported to be the dominant sucrose-cleaving enzyme in this region (Irving and Hurst 1993). The results obtained in the present study showed that spear tips had a much higher protein content compared to other parts of the spear, indicating an active metabolic status. From spear tip to elongation zone, a region undergoing rapid cellular elongation, AI activity increased four-fold, whereas SS remained essentially constant.

The sharp rise in AI activity, which coincided with a decline in sucrose content and a sharp rise in hexose content, strongly suggests an important role for AI in spear elongation growth. From the elongation zone to the base of the spears, AI activity declined and SS activity increased as cell elongation ceased and lignification of the vascular tissue began. The balance between the decline in AI activity and increase in SS activity, together with the gradual decline in lignifying processes (Culpepper and Moon 1939a), is consistent with the hexose accumulation in the base section of spears. The increased activity of SS in the basal region suggests that it may play a major role in sucrose cleavage, possibly in the supply of nucleotide sugars for biosynthesis of cellulose in the rapidly lignifying tissue (Irving *et al.* 1997). The loss of AI activity alone may not necessarily lead to an increase in sucrose content in the base of spears. There are two possible reasons for this. Firstly, the reaction catalyzed by SS is readily reversible (King *et al.* 1997). Secondly, Alam *et al.* (1999) have presented evidence that sucrose accumulation in the base of spears is associated with an increase in both SS and sucrose phosphate synthase (SPS) activities. If this is the case, SPS may play a role in regulation of the ratio of sucrose to hexose. Thus, sucrose and hexose accumulation may be determined by the balance between sucrose synthesis (SPS activity) and degradation (AI and SS activities) as well as the utilization of sucrose in the synthesis of spear structure and storage materials.

It is evident from these results that apart from sucrose cleaving enzymes, the associated biochemical processes for structural and component synthesis in spear tissues also contribute to the regulation of carbohydrate accumulation. Clearly, there are significant changes in carbohydrate partitioning patterns and enzyme activities along the length of developing spears. It is most likely that carbohydrate metabolism in spears is a whole-spear property influenced by both hexose formation and consumption.

5.5 Summary

To assess the relative importance of sucrose cleaving enzymes in the regulation of carbon accumulation in developing asparagus spears (growing shoots), spear elongation, carbohydrate accumulation and enzyme activities of acid invertase (AI), neutral invertase (NI) and sucrose synthase (SS) were investigated in this chapter. The greater elongation rate measured in the high-yielding cultivar ASP-69 was associated with a significantly higher hexose accumulation ($P < 0.05$) in spear tissue in comparison with the low-yielding cultivar ASP-03. However, sucrose content was similar in the two cultivars, suggesting a more efficient machinery for transport and catalysis of carbohydrate in spears of ASP-69. Biochemical evidence indicated that the greater elongation rate in ASP-69 was associated with a significantly higher AI activity ($P < 0.05$) in the elongation zone, whereas SS activity was not significantly different between the two cultivars. There was little NI activity detected in either cultivar. These results strongly suggest that it is AI and not SS or NI that is an important determinant of the difference in sucrose metabolism between the two asparagus cultivars in metabolising imported sucrose in the elongation region, which in turn plays a part in regulating the import of sucrose into spear tissue. The profile of sucrose cleaving enzyme activities along spear sections indicated that SS was the dominant enzyme in both the tip and base of spears, whereas AI was the dominant enzyme in the elongation zone. Apart from sucrose cleaving enzymes, the associated biochemical processes for structure and component synthesis in spear tissues have also contributed to the regulation in carbohydrate accumulation. It is most likely that carbohydrate metabolism in the developing spears is a whole spear property influenced by sucrose degradation (AI and SS activities) and its utilisation in building spear structure and storage materials. The overall data substantiate the conclusion that changes in activities of sucrose cleaving enzymes are correlated with sink functions in developing spears.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUDING REMARKS

Chapter 6

General discussion and concluding remarks

6.1 Introduction

Scientific knowledge and understanding of physiological and genetic influences on crop yield have advanced considerably in recent decades (Gifford and Evans 1981; Daie 1988; Wardlaw 1990; Evans 1993; Evans 1994; Sinclair 1994; Lawlor 1995). Great increases in crop yield have been attributed primarily to the plant's ability to partition more assimilates into harvestable organs under progressively improved systems of agricultural input (Wardlaw 1990; Evans 1994). Accordingly, there has been considerable interest in the physiological changes and controls underlying source-sink relationships in crop yield (Foyer 1987; Sonnewald and Willmitzer 1992; Boyer 1996; Daie 1996; Foyer and Galtier 1996; Pollock and Farrar 1996). A body of research concerning source-sink relations has assumed that coordination between carbon assimilation and partitioning is a central regulating feature of these relationships (Michael *et al.* 1990; Wardlaw 1990; Mitchell *et al.* 1992; Foyer and Galtier 1996; Pollock and Farrar 1996; Quick and Schaffer 1996; Lewis *et al.* 2000; Noormets *et al.* 2001).

Evidence in the literature outlines the importance of storage roots of asparagus in the coordination of carbon production during fern growth and its utilization during shoot

development in the following season (Tiedjens 1924; Tiedjens 1926; Downton and Törökfalvy 1975; Benson and Takatori 1980; Shelton and Lacy 1980; Haynes 1987; Pressman *et al.* 1989; Shiomi 1992; Pressman *et al.* 1993; Ernst and Krug 1998; Faville *et al.* 1999a; Krug 1999a; Wilson *et al.* 1999; Woolley *et al.* 1999). However, relatively little is known regarding the physiological mechanisms underlying source-sink relationships, especially the coordination among carbon assimilation and partitioning into storage roots and its subsequent utilization in developing shoots, where spear yield is determined. The objective of this study was to describe and understand the physiological characters underpinning cultivar difference in spear yield of two asparagus cultivars with contrasting yield. Emphasis was directed towards the understanding of carbon assimilation and partitioning and sucrose metabolism.

This objective can be expressed as the questions posed in chapter 1:

With respect to source-sink relationship in asparagus, what are the physiological limits to carbon mobilization into storage roots and remobilization into developing spears, and within these limits, what metabolic processes contribute to cultivar difference in spear yield?

The specific issues concerning carbon assimilation, carbon partitioning and carbohydrate metabolisms have been discussed in detail in the previous chapters. Based on the current study and available information from the literature, the major objective of this chapter is to synthesize the results presented in the body of this thesis and to develop a conceptual physiological model describing the potential physiological limits to spear yield.

6.2 Carbon assimilation and partitioning

6.2.1 Carbon assimilation

Based on diurnal and seasonal changes in carbon assimilation and partitioning characteristics (Chapter 2 and 3), development of cladophyll tissue in asparagus can be divided into four phases: (1) fast-expanding phase; (2) fully expanded phase; (3) maturation phase; (4) senescence phase. The first phase is characterised by a rapid increase in sucrose content and activity of SPS, whereas hexose content and the activities of sucrose cleaving enzymes decline rapidly. The second and third phases are characterised by the greatest photosynthetic rate associated with the greatest activities of rubisco and SPS enzymes. The fourth phase is characterised by a rapid decline in photosynthetic rate accompanied by rapid decreases in rubisco and SPS activities following the onset of cladophyll senescence (when plants experience lower night temperatures and shorter day-lengths.

The greatest cultivar differences in carbon assimilation were observed in the second and third phases at a time when both photon flux density (PFD) and temperature were at a maximum. Smaller differences were observed in the rapidly expanding and senescent phases (Fig. 2.2, Chapter 2). Thus, timing of cladophyll initiation and duration did not appear to be significant factors contributing to cultivar differences in assimilate production. Biochemical analysis indicated a tight coupling between g_s and biochemical capacity for CO_2 assimilation (Chapter 2). Although the correlation between A and rubisco activity did not exist, both *in vivo* and fully activated rubisco activities in ASP-69 were significantly greater than in ASP-03, indicating the important role of this enzyme in influencing cultivar differences in photosynthetic capacity. Apart from physiological differences, variations in photosynthetic capacity between the two cultivars were related to significant differences in cladophyll thickness and specific leaf weight (SLW) (Table 2.5, Chapter 2). These results

substantiate the conclusion that both metabolic and anatomical factors play significant roles in determining differences in photosynthetic capacity between the two asparagus cultivars studied.

6.2.2 Carbon partitioning

Previous work has demonstrated that the patterns of carbon partitioning in mature asparagus plants are relatively simple in comparison to other crops (Tiedjens 1924; Robb 1984; Faville *et al.* 1999a; Woolley *et al.* 1999). After the period of fern establishment, there is little new fern growth and the majority of assimilates are partitioned to storage roots. Although buds have been shown to have a higher priority for current assimilates, the percentage of assimilate accumulated in buds is much less than the storage roots due to their small mass (Tiedjens 1924; Tiedjens 1926; Robb 1984; Faville *et al.* 1999a; Woolley *et al.* 1999). The results from the present study (Chapter 3) are consistent with the findings of Faville (1999a) that storage roots are the major site in partitioning exported assimilates. In addition, a close relationship between the rate of net photosynthesis and the rate of carbon export in mature cladophylls was found, suggesting a significant role of source tissue on carbon partitioning.

6.3 Physiological approaches to source-sink relationships in asparagus

6.3.1 Feed-forward effects on the translocation process

Like most higher plants, sucrose in asparagus is the major product of photosynthesis in source cladophyll tissue (Pressman *et al.* 1993; King *et al.* 1995; Chapter 3 and 4).

It is also the major translocated sugar and substrate for carbon metabolism in developing and storage sinks (King *et al.* 1990; Lill *et al.* 1990; Hurst *et al.* 1993; Chapter 3 and 5). In this context, we may expect that increases in carbon assimilation would have direct effects on the translocation process if the phloem transport property itself did not limit translocatory flux (Wardlaw 1990; Farrar 1992; Thorpe and Minchin 1996), as it is generally accepted that phloem transport involves mass flow driven by a gradient of turgor pressure (the Munch hypothesis; Fig. 6.1.) (Farrar 1992).

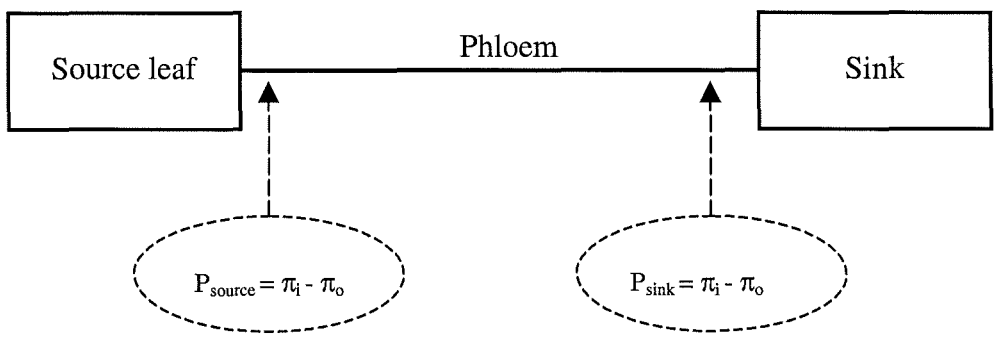


Fig. 6.1. Determinants of carbon translocation rate through the phloem. If the sieve tubes are considered as pressurized vessels connecting a source leaf to a sink, the pressure within sieve tubes in the source leaf, P_{source}, is determined by the difference in osmotic potential (π_i - π_o) across their boundary membrane. Pressure within the sieve tubes in the sink, P_{sink}, is similarly determined. The difference, P_{source} - P_{sink}, gives the driving force for transport through the phloem.

According to Munch hypothesis (Farrar 1992), the loading of sucrose into sieve tubes and unloading in sink tissue creates the major force for mass flow because sucrose is the osmotically dominant solute in sieve tube sap of nearly all plants (Komor 2000). Consequently, sucrose export rate should depend on sucrose availability in the leaves (i.e. Michaelis-Menten-type kinetics as in other active transport processes; Komor 2000). The results obtained in the current study (Chapter 3 and 4) indicate a close relationship between rate of net photosynthesis and rate of assimilate export, This is particularly evident in mature cladophyll tissue, suggesting a feed-forward effect on assimilate export. However, the correlation between sucrose concentration and carbon export did not exist although the high-yielding cultivar displayed a significantly greater sucrose concentration than in low-yielding cultivar. These results suggest that it may not be the sucrose concentration but rather its delivery to the phloem that is important in determining carbon export rates (Mitchell *et al.* 1992). Phloem ^{14}C labelling experiments under both field and controlled conditions provided evidence that greater assimilation rate in ASP-69 may lead directly to a greater ^{14}C flux out of cladophyll tissue in comparison to ASP-03 (Chapter 3 and 4). Similar results have also been reported in other plants. For example, Fader and Koller (1983) reported a positive correlation between photosynthesis rate and export rate in soybean leaves. More recently, Grodzinski *et al.* (1998) examined 21 C_4 *vs* C_3 species by ^{14}C labelling of leaves and found that carbon export rate was closely correlated with photosynthetic rate. However, Both Fader and Koller (1983) and Grodzinski *et al.* (1998) also reported that rate of export was positively correlated to sucrose concentrations in the source tissue. The current finding of an uncoupling between sucrose concentration and carbon export is consistent with findings of Mitchell *et al.* (1992) that it may be sucrose delivery to the phloem rather than the sucrose concentration that is important in determining carbon export rates.

6.3.2 The physiological role of storage roots

There is a considerable body of evidence indicating that photosynthetic performance in asparagus does not directly contribute to spear yield (Downton and Törökfalvy 1975; Robb 1984; Woolley *et al.* 1999). Annually, asparagus plants first allocate a significant fraction of their photosynthetic output to long-term storage roots and after winter dormancy the storage carbohydrates are utilised in spear growth during the next spring (Benson and Takatori 1980; Shelton and Lacy 1980; Woolley *et al.* 1999). However, less information exists regarding the physiological transition from sink to source in the storage roots.

6.3.2.1 Storage roots as a sink

Several lines of evidence in the literature indicate the importance of fructan metabolism in carbohydrate accumulation in storage roots of asparagus (Benson and Takatori 1980; Shiomi 1980; Cairns 1992; Shiomi 1992, 1993). In the current study (Chapter 3), fructan metabolism was not measured directly. However, it is generally accepted that fructan metabolism is positively linked to sucrose content and this relationship is more than merely structural (Pollock *et al.* 1996). For example, feeding ^{14}C to tissue accumulating fructan leads to the initial appearance of radioactivity in sucrose, followed by the progressive appearance of label in fructans. This relationship is quantitative, with all the label initially accumulated in sucrose transferred eventually to fructan (Pollock 1979; Pollock *et al.* 1996). Thus, sucrose content may be taken as an indicator of fructan metabolism (Pollock *et al.* 1996).

In this study (Chapter 3), the two cultivars exhibited similar patterns in TNC and sucrose contents in storage roots. However, following fern establishment, the initial replenishment of TNC was not associated with an increase in sucrose content. The delay in sucrose increase was accompanied by a significant increase in hexose content

and this was more pronounced in ASP-69 than in ASP-03 (Chapter 3). These results suggest that apart from being a source for fructan metabolism, sucrose may also be utilized in root metabolism for root regrowth or for producing new storage roots. This conclusion is supported by the finding that ASP-69 possessed significantly greater young storage roots than in ASP-03 (Chapter 3). Enzymatic studies provided further evidence that SS, not AI, in ASP-69 was significantly greater than in ASP-03 at this stage.

6.3.2.2 Storage roots as a source

There is good evidence that fructan accumulation is localized in the vacuoles of long-term storage sinks (Frehner *et al.* 1984; Carpita *et al.* 1991; Pollock *et al.* 1996). Although not studied as extensively, similar seasonal patterns in fructan content have been observed in different species (Pollock *et al.* 1996). After a period of fructan accumulation in summer and autumn, a mobilization to sucrose generally occurs in later winter, followed by utilization to promote spring regrowth (Pollock 1979; Pollock *et al.* 1996). The results obtained from the current study (Chapter 3) were consistent with the findings of Pollock (1979) that sucrose content increased progressively during winter dormancy. Similar results have also been reported in other asparagus cultivars (Shiomi 1992; Pressman *et al.* 1993). It has been argued that, apart from its function as a major form of translocated carbohydrate and substrate for carbon metabolism, sucrose has regulatory functions (Pollock and Farrar 1996; Chiou and Bush 1998). Accordingly, the increases in sucrose content during the winter season may suggest a physiological role for sucrose to act as a signal for sprouting of the dormant buds (Pollock 1984; Pressman *et al.* 1993; Pollock and Farrar 1996).

6.3.3 Sucrose import and metabolism in developing spears

While spear yield is largely dependent on the availability of resources in the storage roots and the number of buds (Culpepper and Moon 1939a; Benson and Takatori 1980; Robb 1984; Faville *et al.* 1999a; Wilson *et al.* 1999; Woolley *et al.* 1999), there is no indication that carbohydrate reserve has a feed-forward effect on shoot growth. In some species with fructan as a long-term storage carbohydrate, it has been shown that fructan utilization from long-term storage organs is induced in response to a deficiency of current photosynthate supply to the developing organ (Bonnett and Incoll 1992; Schnyder 1993; Pollock *et al.* 1996). In this context, it is developing tissue rather than storage tissue that may be an important determinant of carbohydrate remobilization following spring regrowth. In this study (Chapter 5), greater shoot elongation rates in ASP-69 were associated with greater TNC accumulation, particularly in the elongation and base regions of spears. This suggests that carbohydrate availability for developing spears is greater in ASP-69 than in ASP-03. However, in the storage roots the two cultivars showed no differences in TNC concentration. Thus, ASP-69 must have a greater ability in importing carbon from storage roots than does ASP-03. Measurements of individual carbohydrate components show that hexose content in the spear tissue was greater in ASP-69 than in ASP-03, whereas sucrose content was not significantly different. This may indicate a more effective machinery for transport and catalysis of carbohydrate in spears of ASP-69 (Sung *et al.* 1994; Farrar 1996).

Since the greater hexose accumulation in ASP-69 was not associated with a decrease in sucrose content compared to ASP-03, it is possible that the greater hexose accumulation in ASP-69 was due to greater activities of sucrose-cleaving enzymes rather than the availability of sucrose (Chapter 5). Measurements of enzyme activities revealed that the different utilisation of sucrose in the elongation zone of spears was mainly due to greater AI activity, not SS activity. AI activity in the elongation zone of ASP-69 was generally greater than in ASP-03 except in the early growth stage,

whereas SS activity was not significantly different throughout the growth stages studied. There was little NI activity detected in either cultivar. These results suggest strongly that it is AI and not SS or NI that is an important determinant of the difference in sucrose metabolism in the two cultivars (Chapter 5).

6.4 Whole plant coordination between sources and sinks

As a general rule, source strength must equal sink strength at a whole plant level. This implies that biomass production may be either source or sink limited (Mitra *et al.* 1993; Patrick 1998). In this context, source limited biomass production may occur when the realised rate of assimilate supply from the sources is less than the potential rate of assimilate utilization by the sink tissues. Similarly, sink limited biomass production may occur when the potential rate of assimilate supply from the sources exceeds the realised rate of assimilate utilization by the sink tissues (Patrick 1998). In asparagus, as evidenced in the literature (Tiedjens 1924; Tiedjens 1926; Benson and Takatori 1980; Robb 1984; Faville *et al.* 1999a; Wilson *et al.* 1999; Woolley *et al.* 1999) and extended in this study (Chapter 3 and 5), the increase in genetic yield potential has been directly linked to the size of the carbohydrate reserve pool but not its concentration. Thus, the nature of the storage roots in relation to spear yield is an important element of this thesis.

6.4.1 Adjustment of sink strength to available resources

Since asparagus yield is largely dependent on the amount of carbohydrate reserve partitioned to the storage roots and the number of buds that are capable of developing into spears (Tiedjens 1924; Benson and Takatori 1980; Haynes 1987; Wilcox-Lee and Drost 1990; Wilson *et al.* 1999; Woolley *et al.* 1999), an efficient allocation of assimilated carbohydrate into storage roots may be critical to ensure carbohydrate

reserves are available for spear development. In this study, ASP-69 not only had a greater total biomass but also a greater root/shoot ratio than ASP-03, whereas carbohydrate concentration in storage root tissue was not significantly different between the two cultivars. Allocation of carbon in favour of dry matter increase rather than an increase in carbon concentration in the storage roots may partly contribute to greater spear yield in ASP-69. These results are consistent with those of Benson and Takatori (1980) who reported that a high-yielding cultivar had a higher root/shoot ratio than a lower-yielding cultivar. A similar result has also been reported by Wilcox-Lee and Drost (1990), who suggested that differences in yield between asparagus cultivars might be related to differences in the patterns of carbohydrate distribution within the plants. These results suggest that sink capacity rather than sink activity is responsible for attracting incoming assimilates and preventing any feedback effect on carbon partitioning. Changes in SS activity in storage roots observed in this study were consistent with changes in hexose content during the fern growth season. In this regard, the rise in SS activity may be related to the unloading of sucrose translocated from the shoots or regrowth of storage roots, since it has been reported that SS activity is usually associated with sink demand (Sung *et al.* 1989). Thus, both SS activity and the size of the carbohydrate pool exert a significant influence on carbon partitioning. Such events, if frequent, could greatly influence plant carbon budget and whole plant allocation patterns as observed in some perennial species (Canadell and Lopez-Soria 1998).

6.4.2 Carbohydrate storage and utilization

Evidence from the literature (Pressman *et al.* 1993; Faville *et al.* 1999a) and also from the current study indicates that respiration during the winter dormant period is the primary consumer of stored carbohydrates. In a temperate climate, total carbohydrate reserves in storage roots are relatively stable during winter dormancy, indicating asparagus plants remain metabolically dormant. After winter dormancy, shoot emergence accounts for the major fraction of carbon consumption during spring,

particularly in its earlier stages. The importance of storage root carbohydrates to shoot growth is indicated by the reduced shoot diameter and height in both cultivars following cladophyll shading during the previous season (Table 3.1, Chapter 3). Shading caused a significant decrease in carbohydrate content in storage roots. However, the amount of carbohydrate present in storage roots at the end of fern establishment in the second season did not differ between shaded and unshaded controls, indicating that the remaining carbohydrates are not available to the plant for withdrawal.

Although spear yield is largely dependent on the amount of available carbohydrate reserves in the storage roots (Benson and Takatori 1980; Robb 1984), spear extension growth appears independent of carbohydrate concentration in the storage roots (Chapter 5). This was indicated by the finding that ASP-69 exhibited a greater shoot elongation rate than ASP-03, whereas carbohydrate concentration in the storage roots was not significantly different. Greater shoot elongation rate in ASP-69 than in ASP-03 was attributed to greater AI activity in the elongation region. According to the physiological unit concept that carbohydrate production and allocation in asparagus plants is confined to individual rhizomes and associated roots and shoots (Hughes 1992; Faville *et al.* 1999a; Woolley *et al.* 1999), it is likely that shoot development in asparagus is influenced by both available carbohydrate reserve in the source storage roots and sink activity to import carbon for development.

6.5 A conceptual model

Having achieved the results reported in chapter 2, 3, 4 and 5, together with the available information from literature, the aim of this section is to outline a conceptual model (Fig. 6.2.), which describes the physiological limits to asparagus yield in terms of carbon production, storage and utilization. This section should be treated as an extension to the original hypothesis, and will concentrate on carbohydrate

mobilization and related metabolisms. It is not intended to imply that the other aspects of physiology and environment are less worthy of attention.

6.5.1 Carbohydrate utilization in developing spears

As indicated in Fig. 6.2 (1), spear yield is dependent on the availability of carbohydrate reserve in the storage roots and the number of buds capable of developing into spears. In this context, any influence on carbohydrate utilization and bud germination will affect spear yield. Results from the current study indicate that apart from the availability of root storage carbohydrates, sink activity also contribute greatly to spear elongation growth (Chapter 3 and 5). Enzymatic analysis suggests that AI is an important determinant of differences in metabolising imported sucrose in the elongation region, which in turn plays a part in regulating the import of sucrose into spear tissue. Thus, although both source supply and sink demand are related to spear production, they influence spear yield in different ways. Source supply determines the amount of available carbohydrate for spear uptake, whereas its utilization is likely determined by sink demand in developing spears.

6.5.2 Shoot establishment

It has been frequently highlighted that early emerging asparagus plants generally show a greater yield than late emerging plants and fern vigour is generally correlated with spear yield (Ellison and Schermerhorn 1958; Ellison *et al.* 1960; Robb 1984). In the current study, the two cultivars did not differ in the period of photosynthetic production. However, ASP-69 had a greater shoot size, associated with greater sucrose cleaving enzyme activities in immature growing tips in comparison to ASP-03 (Chapter 3). These results indicate that spear yield and shoot size were closely

coupled. Greater canopy size ensures greater total assimilate production (indicated in Fig. 6.2 (2)).

6.5.3 Feed-forward effect on carbon export

A close relationship between the rate of photosynthesis and spear yield has been reported on both a cladophyll and canopy level (Woolley *et al.* 1996; Bai and Kelly 1999; Faville *et al.* 1999b). The current study confirms the finding of Faville *et al.* (1999b) that A_{max} is positively associated with asparagus yield. In addition, a close relationship between photosynthesis, sucrose synthesis and assimilate export rate in the source cladophyll tissue was found (Chapter 2 and 4) indicating a feed-forward effect on assimilate export. This is highlighted in Fig. 6.2 (3). In this regard, asparagus yield is likely source limited rather than sink limited in terms of carbon partitioning into storage roots.

6.5.4 Carbon partitioning into storage roots

As indicated in Fig. 6.2 (4), the majority of the assimilated carbon is translocated into the crown, except in the fern establishment period in which a significant fraction of assimilate is allocated into growing shoots (Woolley *et al.* 1999) After the fern has fully established, the storage root fraction is the dominant sink for current assimilates throughout the fern growth season (Benson and Takatori 1980; Haynes 1987; Pressman *et al.* 1993; Faville *et al.* 1999a). Greater spear yield in the high-yielding cultivar is attributed to a greater root/shoot ratio (Benson and Takatori 1980; Wilcox-Lee and Drost 1990; also in Chapter 3). This assumption is supported by the present findings that the high-yielding cultivar shows a greater percentage of young storage roots, associated with a greater sucrose cleaving enzyme (sucrose synthase) activity, whereas carbohydrate concentration was not significantly different between the two cultivars (Chapter 3).

Although only a small fraction of storage carbohydrate and current assimilates are utilised in new bud and new storage root production, it is critical for spear production in the next season. In this context, breeding for partitioning in favour of new bud and storage root production will likely lead to an increase in spear yield.

6.5.5 Conclusion

Taken together, this conceptual model provides a framework of the annual carbon cycle of mature asparagus plants and illustrates that spear yield in asparagus is a property influenced by both source and sink properties. This is highlighted by the fact that spear elongation is related to spear ability to import carbon and overall yield is determined by available carbohydrate reserve in storage roots which in turn is related to assimilate production.

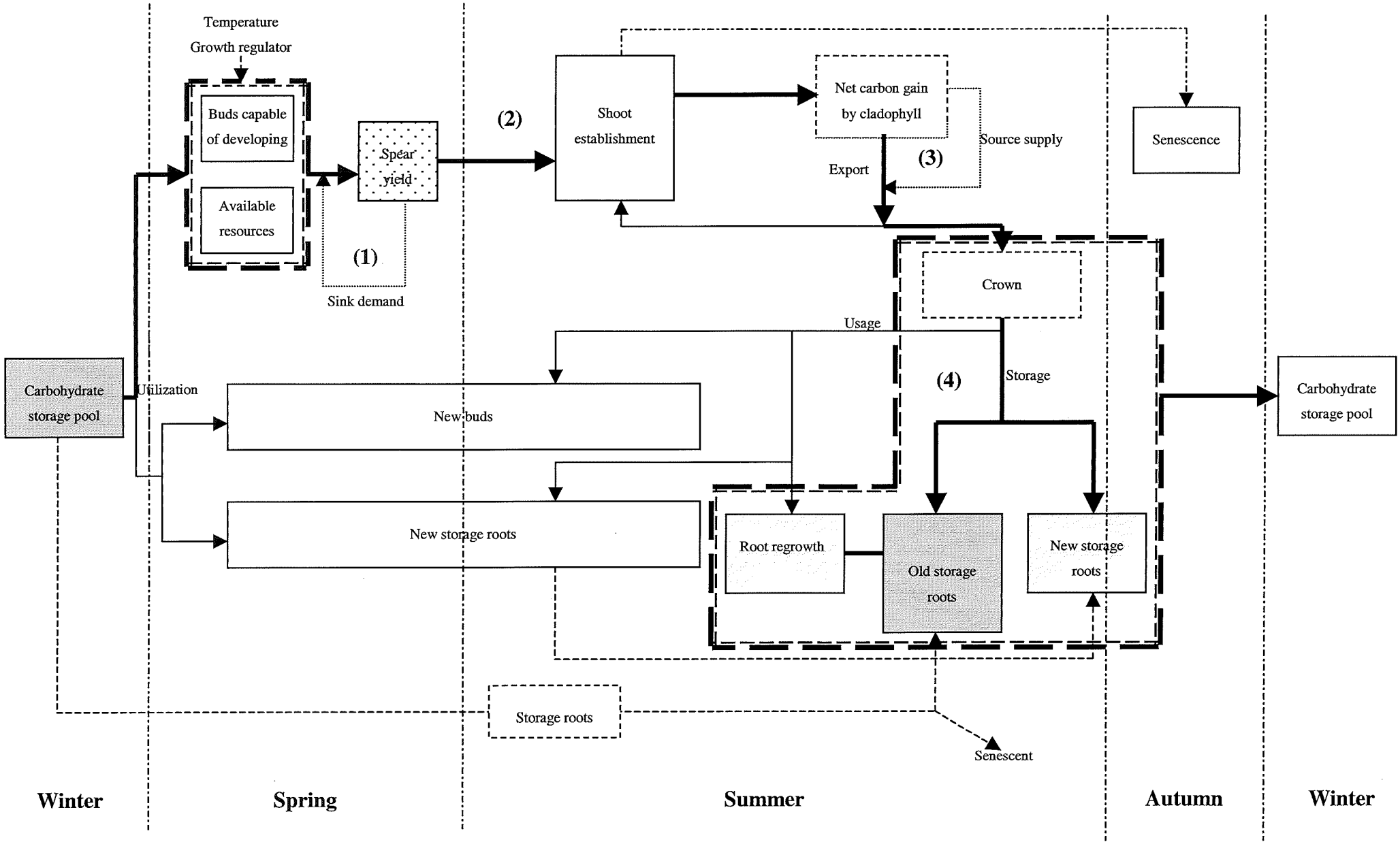

Temperature
Growth regulator
Buds capable of developing
Available resources
Spear yield
(1)
Sink demand
(2)
Shoot establishment
Net carbon gain by cladophyll
Source supply
Export
(3)
Senescence
Crown
Usage
Storage
(4)
Carbohydrate storage pool
Utilization
New buds
New storage roots
Root regrowth
Old storage roots
New storage roots
Carbohydrate storage pool
Storage roots
Senescent
Winter
Spring
Summer
Autumn
Winter

Fig. 6.2. A conceptual model of source-sink relationships in relation to asparagus yield. Emphasis is given to carbon assimilation, partitioning and utilization during an annual life cycle of a mature asparagus plant. It is based on both the literature and the current study. The two dark dash-line enclosed sections indicate sinks in the storage of assimilated carbon and sources for spear yield, respectively.

6.6 Future prospects

World asparagus production is continuously increasing in order to meet worldwide increased consumer demand (Benson 1999). However, statistics have shown that increase in asparagus production is primarily a result of increase in growing area rather than increase in yield per plant (Benson 1999). Consequently, maximising cultivar productivity has been the subject of extensive research in breeding practice (Nichols, 1990; Benson, 1999). It is acknowledged that selections for spear yield among asparagus cultivars based on agronomic characters are quite costly and unreliable (Nichols and Woolley 1985). Thus, to achieve the goal of creating new varieties with high yield and genetically stable characters, we need to have a sound understanding of yield physiology. Unfortunately, very limited information is available regarding the physiological basis underpinning cultivar differences in spear yield.

In recent decades, major changes in asparagus production have been attributed to breeding programmes which develop clonal hybrids and all-male varieties (Nichols 1990; Benson 1999). Many of these new varieties have been shown to possess both increased yield and long-term genetic stability (Robb 1984; Mullen *et al.* 1996; Smeenk *et al.* 1996). Using all-male clonal cultivars to investigate physiological differences in relation to asparagus yield, the current study demonstrates a feed-forward effect of photosynthesis on carbon export rate out of source tissue via its effect on sucrose synthesis. Furthermore, it is the total amount of storage carbohydrate reserve rather than carbohydrate concentration that is the important determinant of asparagus yield. In addition, carbohydrate availability is related to the overall spear yield, but not spear elongation rate. In this context, a further increase in spear yield may be achieved by breeding for specific carbon assimilation or partitioning type cultivars. This might be achieved using a number of strategies:

- It has been shown that after the initial establishment stage (1-2 years), asparagus plants tend to increase root/shoot ratio until plants are mature (5-6 years) after which the root/shoot ratio is relatively stable and balanced by the senescence of old roots and production of new roots (Blasberg 1932; Moon 1976; Fisher 1982; Douglas 1990; Hughes *et al.* 1990; Stancanelli and Falavigna 1990; Feher 1992; Krug 1996; Ernst and Krug 1998). In this regard, a close relationship between assimilate production and its allocation into storage roots may be expected as sink strength in carbohydrate storage is unlikely a limit factor. Indeed, greater shoot biomass is associated with greater root biomass in the two cultivars studied (Chapter 3). In addition, the high-yielding cultivar in this study also displayed a greater A_{max} than the low-yielding cultivar, indicating that asparagus may be source limited rather than sink limited in the relationship between assimilate production in cladophylls and storage in the storage roots. Accordingly, a further increase in asparagus yield may be expected by an increase in total assimilate production. It is likely that, apart from the limitation from canopy level, spear yield can be partly limited by the photosynthetic capacity on the cladophyll level, especially in the fully expanded and mature cladophyll tissue at a time when both PFD and temperature are at a maximum. Thus, selection of high-yielding cultivars with greater cladophyll A_{max} associated with an increase in canopy photosynthesis may have potential to increase total assimilate production, Further investigation is needed to confirm the relationship between A_{max} and spear yield in a wide range of asparagus cultivars. CO_2 enrichment experiments could provide new insights into the feed-forward effect of carbon assimilation on carbon export.

- Maximising storage root sink strength would be another practical way to increase yield potential as it is related to the overall spear yield and the subsequent fern establishment that determines yield in the next season. To achieve this, selection of varieties with a high percentage of new storage root production and regrowth of storage roots will be essential. Breeding for partitioning in favour of new bud and storage root formation is critical to potential bud size and emergence. Further research is needed to elucidate the mechanism by which new storage roots are initiated and the stimulus that triggers the event. The role of sucrose in regulating sink and source

activities and its effect on carbohydrate translocation needs additional study. The study of whole plant physiology on the canopy level in relation to carbon budget and spear yield is also needed. 2-3 year-old plants may prove to be an excellent system in which to conduct plant level experiments. Root growth in comparison to shoot growth under various circumstances needs to be more fully understood.

- Study in carbohydrate metabolism of developing spears indicated that there was a considerable increase in reducing sugars in the high-yielding cultivar compared to the low-yielding cultivar, whereas the sucrose content was similar, suggesting a role of sink regulation in carbohydrate reallocation into developing spears. Measurements of enzyme activities indicated that this difference was mainly due to greater acid invertase activity in the elongation region of the spear. Future research is needed to determine the universality of the above findings and to shed light on the relative contributions and control of competing and coexisting metabolic pathways and the importance of tissue and cellular localization of pathways.

Comparison based on the two selected cultivars indicates a close relationship between spear yield and carbon assimilation, utilization and metabolism, suggesting cultivar variations in photosynthetic capacity and carbon metabolism may be linked to differences in spear yield in asparagus. However, crop yield among species or cultivars under field conditions is a complicated process and regulated by both genetic and environmental components. Although photosynthesis is the ultimate determinant of plant productivity, photosynthetic capacity is only one aspect of plant production. The leaf area index (LAI) or the canopy photosynthesis have been shown to be more important determinants of plant production. The total net photosynthesis depends on the integrated activities of all photosynthetically active organs and responses of photosynthesis of individual leaves to radiation. Thus, further work on canopy photosynthesis is needed for confirming the broad validity of conclusions made in this study to asparagus in general and also for estimating the extent to which yield

differences between asparagus cultivars are due to differences in cladophyll photosynthetic capacity.

6.7 Concluding remarks

Spear yield in asparagus is a property of the entire plant system. It involves the balance between photosynthetic carbon gain in cladophylls and subsequent assimilate partitioning into the root system during the fern growth season as well as an efficient reallocation into spear components after winter dormancy. In source cladophyll tissue, a close relationship between A_{max}, and assimilate export was found, suggesting that asparagus yield is source limited in terms of carbon assimilation and partitioning. Storage roots play a significant role in regulating carbohydrate partitioning and availability for spear yield, but do not determine its utilization during spear development. This is highlighted by the findings that the high-yielding cultivar displayed a greater root to shoot ratio compared to the low-yielding cultivar, accompanied with great sucrose synthase activity, whereas the root carbohydrate concentrations were not significantly different. Biochemical analysis in the developing spears indicated that utilization of storage carbohydrate during spear growth is primarily determined by sink demand rather than source supply. This is indicated by the fact that high-yielding cultivar displayed greater acid invertase activity in the elongation region of developing spears. It is concluded that spear yield is influenced by both source and sink properties in which spear elongation is closely related to spear ability to import carbon and the overall yield is determined by the available carbohydrate reserve in storage roots, which in turn is linked to the assimilate production.

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